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THE TIME-LAW OF INTERSEXUALITY

by

RICHARD GOLDSCHMIDT

University of California, Dept of Zoology

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A few years ago (1934) I published an extensive review of my work on intersexuality in *Lymantria dispar*, with which I considered my

own contributions to this subject as closed. Therefore I am returning only reluctantly to the same topic. It has however become necessary because recently the basic conceptions, derived in explanation of the phenomenon which I had called intersexuality, have been criticized by two authors, KOSMINSKY and BALTZER. The first derived his criticism from the study of an abnormal case which he had found in *Lymantria* itself. BALTZER tried to extend his analysis of the sex-intergrades in *Bonellia* to *Lymantria*, and to do this he had to show that my explanation did not cover all the facts. As it would be very difficult for one not acquainted with the material itself to find out what is wrong with the conclusions of these authors, I feel the obligation to show in detail that their criticism has resulted in failure in order to prevent the spreading of confusion. The material upon which my argument is based is found in the numerous papers which have been reviewed in the summary of 1934, where also the complete bibliography is to be found.

A. INTRODUCTION

In 1930 KOSMINSKY and GOLOVINSKAJA published two papers in which they reported upon the behavior of some Russian strains of *Lymantria dispar*, which behaved very differently from the type, as established in the author's experiments. In innumerable crosses with a variety of geographic races of the gypsy moth it had been established by the author that female intersexuality is produced by crossing the female of a weak race with a male of a strong race. Male intersexuality, however, appears only in the strong female line when a strong F, inherited within the cytoplasm, is combined with two weak M within the X-chromosomes. There are a few minor exceptions to the last rule which have been discussed in the author's papers. KOSMINSKY's surprising result was the following: All the Russian races which he tested — many of them which he had kindly sent to me were also tested by myself with identical results — behaved as weak or half weak races. Crossed with strong males they produced nothing but males (weak mother) or high grade intersexual females (half weak mother). In the reciprocal crosses, which I made, intersexual males appeared in F_2 according to expectation. There is then no doubt that the F of these Russian races is a weak F

But among the normal lines from the localities Woronesch and Poltawa and their crosses, which ordinarily were normal over many generations, three individual exceptional broods were obtained. I emphasize the word exceptional because these broods fall completely out of line also in KOSMINSKY's crosses. As he followed up these exceptional broods and did not further mention their normal sister broods, the superficial reader might gain the impression that the behavior of these broods was a typical one and that KOSMINSKY's results, therefore, did not corroborate mine. Already some biologists have made this mistake. In fact KOSMINSKY had typically the same genetic results with the Russian weak and half weak races as myself. But in addition to this bulk of harmonic results three exceptional broods appeared. Their unique feature — unique as opposed to thousands of crosses with the typical result performed by myself and others — is that male intersexes and broods with only females appeared within the weak female line in different combinations. As a matter of fact, these male intersexes were morphologically different from typical male intersexes, but as they appeared in a way closely paralleling that of real male intersexes they were treated as such. These three broods and their offspring then could not be understood with the genetic assumptions which explained the thousands of other crosses perfectly.

When these papers appeared I felt that some other phenomenon but intersexuality must be involved, some abnormal chromosome behavior leading to a mosaicism resembling intersexuality without being it. But I was unable to furnish a proper genetic explanation, as little as KOSMINSKY could. Unfortunately I later made the mistake of changing this opinion and must therefore plead guilty to my share in the present confusion. Starting from the explanation of some comparable cases I found an explanation on the basis of actual intersexuality. As I had read only the papers which the Russian authors had published in German and which contained only a short account of the results, I was led to assume that the crosses within the three abnormal lines could be explained within the general scheme of intersexuality and therefore assumed that KOSMINSKY's "intersexual males" from these strains were actually intersexes, though somewhat peculiar ones. In a series of recent papers Kosminsky has shown that this, my interpretation, does not agree with the facts contained in his

Russian publication. He contends that he had a case of typical male intersexuality and as these three exceptional lines do not conform to the laws found otherwise he concludes that those laws must be wrong and he devotes a series of papers to prove this point. Assuming even that the three lines studied by KOSMINSKY actually contained male intersexes, it would be a strange procedure to try to explain away the rules found in an immense bulk of perfectly consistent crosses by the abnormal behavior of three lines (these three in addition being exceptions in a series of otherwise typical ones). The proper method, I think, is to find out why such exceptions occur, what they mean and how they fit into the typical course of events. Through the courtesy of Professor E. B. BABCOCK I was able to enlist the services of the translator in his department Mr. I. L. BASS, who kindly furnished a literal translation of the whole set of Russian papers. This enables me to return now to the subject and to discuss KOSMINSKY's results in detail. I shall try to show that my first views on KOSMINSKY's three exceptional lines were correct, namely that no typical intersexuality is involved at all. The so-called intersexes in KOSMINSKY's broods are in fact mosaics, different from intersexes in morphology and development and caused by a different genetic situation. Hence the entire set of criticisms of the theory of intersexuality which KOSMINSKY derives from his abnormal lines is unwarranted.

We shall first discuss the genetic side, afterwards the morphological side and the so-called time law of intersexuality.

B. GENETICS OF KOSMINSKY'S MOSAIC LINES

a. *The three exceptional broods*

It must first be emphasized again that the three abnormal broods were exceptions to otherwise perfectly typical behavior. KOSMINSKY analysed a large number of races from different Russian localities, namely Woronesch, Poltawa, Maikop, Odessa, Bochara, Samarkand, Fergana in different combinations among themselves, in pure strains and by crossing to strong Japanese races. This was done over a number of generations and the results obtained proved that these races all behaved in the

way typical for weak and half weak races. This means that all crosses within these races give normal offspring, that females of these races crossed to strong Japanese males give in F_1 either only males or males and intersexual females. The reciprocal cross Japanese ♀ × Russian ♂ is normal and in F_2 a certain percentage of intersexual males appears. Most of these Russian races have also been bred by myself over many generations. They were used for many different crosses, reported in detail in my papers, which all gave the result typical for weak races. There is in my records a single exception, where in a combination with Bochara and a Korean race, both half weak, a few male intersexes appear.

Thus the numerous crosses and pedigreed broods, both by KOSMINSKY and myself always gave results in full conformity with the whole of my work on intersexuality, establishing the Russian races as typical weak or half weak races.

We come now to KOSMINSKY's three exceptions which he thinks sufficient to overthrow the whole structure of the analysis of intersexuality based upon thousands of consistent crosses. I shall have to report the results of the Russian author in more detail, not only on account of the linguistic difficulties, but also because the results have been presented in a way which makes an analysis very difficult, actually leaving all the analytical work to be done.

The three exceptional broods were the following:

1. Within the pure race from Woronesch one brood appeared containing 32♀ 4♂ 3♂ Mo (♂ Mo means mosaic males. These are the males which resemble intersexes and are called intersexes by KOSMINSKY.)

2. In a cross between a Poltawa ♀ and a Woronesch ♂, both from normal lines, once an abnormal result appeared: 27♀ 5♂ 5♂ Mo.

3. Another cross of the same type gave 39♀ 5♂ Mo.

These three lines were used for further experiments. Unfortunately KOSMINSKY took it for granted that the hereditary behavior of these exceptional broods must fall in line with the typical cases of intersexuality. Therefore he made only a few F_2 and F_3 crosses, but crossed into these lines many other Russian races. This makes the work not very transparent, but, though a complete analysis of the results is hardly possible, the material suffices to show at least the direction of an analysis.

b. Proof that the F/M balance is not involved

The decisive point is to show that these lines do not behave genetically as intersexual lines do. The first decisive proof that something else than intersexuality is involved has been furnished by KOSMINSKY, though this is not his interpretation. (I made the mistake myself, as mentioned, of trying to explain these facts on the basis of intersexuality). He showed that in crosses of females from these abnormal lines with strong Japanese females F_1 gives only males plus a few highest grade intersexual females. This shows that in these abnormal broods the females still behave as weak females, contain a weak F inherited in the cytoplasm. This suggests already that the mosaic males have nothing to do with typical intersexuality. In fact KOSMINSKY was able to combine broods in which the weak F from these broods was combined in some segregants with a strong Japanese M and in others with two weak M from those lines, not mentioning such factors as determine the mosaic males in the exceptional broods, outside of F and M. The result, was that intersexual females and mosaic males occurred within the same offspring. In cases of real intersexuality never do female and male intersexes appear in the same brood, as the former require a weak and the latter a strong M derived from the mother. Thus it is shown that, from the genetic standpoint the mosaic males are suspected of not being intersexes, as this would require an F from the mother which is simultaneously weak and strong. Of course there remain two other possibilities that these mosaics are intersexes. 1. It could be possible that an ultra weak M would give male intersexes with a weak F. I tried this solution, but, as KOSMINSKY correctly criticises, the details of his crosses, as given in the first Russian paper, do not agree with such an assumption. 2. It could be possible that one or more modifying genes exist which enhance the action of the weak F to make it act like a strong F in the male combinations. This would be an action of the type of the T factor analysed in my work on intersexuality. KOSMINSKY is inclined to take this as a part of the solution. In his last contribution, however, he takes refuge in the *deus ex machina* multiple sex genes, without being able to explain a single concrete cross on this basis. We shall show later that the morphology of the mosaics shows that they are different from intersexes. The facts thus far mentioned agree with this.

We come to the second genetic proof that in KOSMINSKY's abnormal lines another phenomenon than intersexuality is involved. KOSMINSKY over and over again stresses the point that genetically the mosaic males are produced like the intersexual males, namely by the combination of a definite F with two definite M. This F is inherited maternally and is always derived from females of one of the three abnormal broods, carried on within the female line. As a matter of fact the proofs that only the maternal line i.e. a female determiner within the cytoplasm is involved, are very meager. He crossed sixteen mosaic males from different broods of the abnormal lines to normal weak females. The offspring was normal. From these he obtained altogether 4 normal F_2 . Of course, if intersexuality of the usual type is involved no male intersexes are to be expected in the typical weak maternal line. But let us suppose, for the argument's sake, that the mosaic males are produced if one gene A is present in homozygous condition and one gene B heterozygous i.e. AABb. F_1 of such a male with a female aabb would be $\frac{1}{2}$ AaBb $\frac{1}{2}$ Aabb. The latter individuals bred among themselves could never produce mosaic males. The few crosses made, then, do not prove anything, whether a maternally inherited F is involved in the production of the mosaic males or not. Therefore no proof may be derived from this set of facts regarding the intersexual status of these mosaics.

But there is also positive proof contained in KOSMINSKY's data that his mosaic males are not produced in a way typical for intersexuality. One of the rules regarding intersexuality is that one and the same combination of F and M gives always the same result, with a small modicum of variation. For example, a strong F combined with two weak M of the race Hokkaido gives only females. This combination has been repeated innumerable times with the same results. Or a strong F with two weak European M will give male intersexes in the presence of the modifying factor T, their percentage depending upon the recombination of T (0-100%). Again this result does not know of exceptions. Or a strong F with one M from Hokkaido, the other from another weak race, gives either only females or females plus a very few high grade intersexual males.

This universal rule, found without exception in innumerable crosses made by myself and others (see *Lymantria*, 1934) however does not apply to KOSMINSKY's mosaics. Here are a few data from his tables:

1. $19 \text{ } \varphi \text{ } F_{\text{Polt } 96} \text{ } M_{\text{Wor no}}$ were crossed to normal Woronesch males. ($F_{\text{Polt } 96}$ means F derived from the maternal race Poltawa; this was crossed to a male from a normal Woronesch line and all daughters therefore were $F_{\text{Polt}} \text{ } M_{\text{Wor no}}$. This F_1 called No. 96 was one of the abnormal broods as reported. The females of this brood of said formula were mated to Woronesch males of a normal line.) Whatever now the result in this set of crosses into the abnormal line, it must be the same in all 19 cases, if the rules for intersexuality hold, namely:

$F_1 \text{ Polt} \times \text{Wor}$ gives $\varphi F_{\text{Polt } 96} \text{ } M_{\text{Wor}} \text{ } \sigma F_{\text{Polt } 96} \text{ } M_{\text{Polt}} \text{ } M_{\text{Wor}}$
 $RF_2 (\text{Polt} \times \text{Wor}) 96 \times \text{Wor}$ gives $\varphi F_{\text{Polt } 96} \text{ } M_{\text{Wor}} \text{ } \sigma F_{\text{Polt } 96} \text{ } M_{\text{Wor}} \text{ } M_{\text{Wor}}$.

The males therefore as well as the females are expected to be identical in all RF_2 crosses and only of one type, (but possibly different from the F_1 males). These 19 crosses in 1927 had the following result:

	φ	σ	3Mo
1.	17	—	—
	25	—	—
	46	—	—
	6	—	—
2.	26	—	4
3.	11	2	1
	24	3	8
	13	5	1
	8	3	2
4.	21	15	6
5.	22	20	4
	31	31	3
6.	21	29	—
	9	13	—
	2	3	—
	1	1	—
	3	2	—
	3	1	—

Even if we leave out of account the small broods, we find not less than 6 different types among these 19 broods, namely:

1. Only females
2. Many females, a few ♂Mo (like F_1 !)
3. Many females, few males, these partly normal, partly mosaic.
4. One half females, one half males, males partly normal, partly mosaic.
5. Females and normal males 1 : 1, a few additional mosaic males.
6. Normal sexes.

There is nothing in the whole field of intersexuality which could compare with this result. It shows beyond any doubt that in the production of the mosaic males in the abnormal line no F/M ratio is involved. It shows, further, that the abnormal result is the consequence of a recombination of something which has nothing to do with F or M, but must be contained in the autosomes as both mothers and fathers must have had different constitutions in regard to this same thing, in order to give such a segregation.

This set does not stand alone. Numerous females from the broods just mentioned, which all must have been $F_{\text{Polt 96}} M_{\text{Wor}}$, were crossed with other males. There are 17 such females crossed with Odessa males. Again all sons must have the same constitution in regard to F and M. Among these broods, all of them with large numbers, we find

1. Only females and mosaic males in about equal numbers.
2. Many females, a few mosaic males.
3. Females, males, and mosaics.
 - a. Many mosaic males, a few normals.
 - b. Large numbers of both, either equal or more normals or more mosaics.
 - c. Among these:
 - a. equal number of sexes
 - b. fewer males in different proportions
 - c. many females, a few males and mosaics.

Further, numerous sisters of one of the broods containing only females were mated to males of another race (Bochara, Fergana, F_1 Woronesch \times Fergana, etc.). Again where the fathers were themselves no hybrids, identical results of all crosses were expected, if intersexuality is involved. In fact we find:

Cross: sisters from only female brood 1927 \times Bochara ♂

Offspring:

- a. only Females
- b. females and mosaic males 1 : 1
- c. females and male Mo, a few males
- d. many females, few ♂ Mo and ♂.
- e. many females, few ♂ Mo.

The same types are obtained with Fergana males and Wor \times Ferg hybrid males.

Going over the whole material we realize (1) that crosses involving the same F and M give very diversified results; (2) that the types appearing are different according to the paternal race.

c. Possibilities

There is then no doubt whatever that genetically the three abnormal lines are completely different from all the crosses involved in intersexuality and that the production of the mosaic males in these lines is in no way genetically comparable to the production of male intersexes. Only two conclusions may be derived from this: Either there exist two different ways of producing male intersexes in *Lymantria*. The first is the one found in the bulk of work with this form, namely when the balance F/M is in favor of F in the combination of strong F and weak M's, with or without presence of the modifier T. The second type is, thus, far, found only in the three abnormal broods of KOSMINSKY. Here F and M are obviously not involved and the phenomenon occurs therefore within a weak race, or crosses of two weak races, or crosses of weak and strong races. It will be shown later that recombinations of autosomal genes are involved.

A priori this alternative does not offer any difficulties. It is known, for example, that in *Drosophila* intersexes are usually produced by a disturbance of the F/M balance in triploid intersexes (BRIDGES) and these intersexes are morphologically strictly comparable to the *Lymantria* intersexes (the time law, DOBZHANSKY). But there exists also in *Drosophila* two other types of intersexes, morphologically different from the triploid intersexes and each other, and caused by a single autosomal gene. (STURTEVANT, LEBEDEFF). It is obvious that if sex is controlled by the F/M balance this may be changed either by a up-

setting of the ratio through a change in F or M, or by any other gene which acts so as to interfere with the effects of this ratio (of course also corresponding external agencies, temperature, hormones, etc.). Only from the first cases (F/M balance) inferences upon the primary sex-determining mechanism can be drawn, as has been done by myself and also by BRIDGES. From the latter cases we can only draw conclusions upon possible modifications, genic or otherwise, of the effects of the primary mechanism. It would greatly help the discussions upon the sex problem if authors would try to have clear notions in regard to these elementary principles.

We shall later see that KOSMINSKY's so-called intersexual males are as different from typical male intersexes as LEBEDEF'F's *Drosophila* intersexes from the typical ones. The question then arises whether or not — this is the second alternative — a different type of sex-intergrade is involved. We know thus far gynandromorphs and intersexes, both being clearly defined since my earlier work. But there exist doubtless sex-intergrades which do not clearly fit the definition of one or the other. In some cases a combination of intersexuality with gynandromorphism may be involved (see my discussion of triploid intersexes in moth in my book on sex-intergrades GOLDSCHMIDT 1931). Cases of this type are now being investigated by SEILER, who may succeed in pushing the analysis to the desirable end. But there are also others which are less clear, for example LEBEDEF'F's *Drosophila* intersexes, STURTEVANT's very different *Drosophila* intergrades. To this category belong also the mosaics bred by KOSMINSKY. It is a question whether the term intersexes ought to be used for such cases. I do not think so. This is just what leads to wrong conclusions. If these different types do not fit the laws of intersexuality (viz. development with a turning point), authors who call these types intersexes are prone to draw conclusions upon the real intersexes, which are bound to be erroneous. It is, therefore, wiser to call such unanalysed forms sex-intergrades until it is known how they are developed. Such a knowledge might or might not lead to the establishment of other categories of sex-intergrades beside intersexuality and gynandromorphism.

These are then, the two only possibilities for the origin of KOSMINSKY's mosaics: either they are intersexes of a special type produced by a genetic cause dif-

ferent from the one producing genuine intersexes; or they are a different type of sex-intergrades resembling only intersexes and based upon a different genetic situation.

Thus far we have only shown (1) that the Russian races used by KOSMINSKY, and also the three exceptional lines, conform completely to the known laws of intersexuality, when this is involved; (2) that the so-called male intersexes produced in the three exceptional lines are the results of a genetic situation, which shows certain regularities but is absolutely different from the one found in real cases of intersexuality.

The question now arises whether the genetic basis of KOSMINSKY's three exceptional broods and their offspring can be analysed. As a matter of fact a complete analysis is excluded, because the proper experiments have not been performed. From the material presented by KOSMINSKY, however, a general idea may be derived, though the details would still have to be worked out. We mentioned already KOSMINSKY's claim that the mosaic males are only produced in the female lines of the three original broods. This would mean that one of the genetic conditions is a definite cytoplasm or Y-chromosome. But we pointed out that the proofs for this are very meager, if not absent. The hundreds of crosses with the female lines of the three broods are faced only by 16 F_1 with normal females which do not prove anything and only 4 F_2 broods. We pointed out that genetic situations may easily be conceived which give this result without any maternal inheritance being involved. This point, then, the eventual necessity of a definite female line for the production of the mosaics, has to be left open, to say the least, in spite of KOSMINSKY's assertions to the contrary.

d. Attempt at an analysis

Leaving this point, we have to try to find in KOSMINSKY's tables (1929) clues for a genetic analysis, which has not been tried by the author. The material consists of crosses of daughters from the three abnormal lines (or grand-daughters) to their own brothers or to males from different normal weak Russian lines. The following facts may be read from these tables:

1. Four primary types of results occur in such crosses:

- a. normal offspring
- b. only females (eventually a few high-grade male mosaics).
- c. females and male mosaics (eventually a few additional normal males).

d. females, males, and male mosaics.

In the group (b) a few exceptional highly „intersexual” males may occur and similarly in group (c) a few normal males. This type of fluctuation has also been found in intersexuality, and we may therefore treat group (b) as only females and group (c) as only male mosaics.

2. In all groups except (b) the ratio of males and females varies. This variation is not haphazard but very typically the following ratios occur:

- a. 1 ♀ : 1 ♂ (with or without mosaics)
- b. 2 ♀ : 1 ♂ „ „ „ „
- c. ca 3 ♀ : 1 ♂ „ „ „ „
- d. many ♀ : 1 ♂ „ „ „ „

3. When both males and mosaic males occur, different ratios are found. Among these the most frequent ones are:

- a. 1 ♂ : 1 ♂ Mo
- b. 2 or 3 ♂ : 1 ♂ Mo
- c. 1 ♂ : 2 or 3 ♂ Mo
- d. many ♂ : few ♂ Mo or vice versa.

The last group may possibly mean only a transgressing fluctuation from the groups only males or only mosaics.

The following table contains typical examples of these groups taken only from broods with large numbers:

Combination	♀	♂	♂Mo	Type
(Polt 96 × Wor) × Wor . . .	21	29	—	1a, 2a
Wor abn. × Ferg	25	11	—	1a, 2b
(Polt 95 × Wor) ^s	32	10	—	1a, 2c
[(Polt 96 × Wor) × Wor] × Ferg	118	—	—	1b
(Polt 96 × Wor) × [Wor × (Wor × Ferg)] . .	98	—	2	1b

Combination	♀	♂	♂Mo	Type
[(Polt 96 × Wor) × Wor]				
× Boch	52	—	50	1c, 2a
do.	51	2	43	1c, 2a
do.	99	—	37	1c, 2b
do.	107	—	34	1c, 2c
do.	104	1	28	1c, 2c
do.	38	1	33	1c, 2a
[(Polt 96 × Wor) × Ferg]	46	8	8	1d, 2c, 3a
[(Polt 96 × Wor) × Wor]				
× Odessa	84	3	7	1d, 2d, 3c
do.	110	81	24	1d, 2a, 3b
do.	113	29	55	1d, 2a(?), 3c
do.	68	35	17	1d, 2a, 3b
Wor × Maikop	93	22	59	1d, 2a, 3c
do.	67	7	38	1d, 2a, 3d (2)
do.	104	82	6	1d, 2a, 3d (1)

It might be said that the majority of the broods are like these selected examples. Those who show a certain deviation, if added to their nearest group, do not change the mean ratios.

To these general features a few details may be added. We pointed out already that sister crosses which in a normal intersexuality experiment would give identical results always show different types of behavior. The types occurring in a series of identical crosses are obviously dependent upon the races involved. The following examples may be taken from KOSMINSKY'S tables.

1. In the abnormal line 1, derived from a pure Woronesch race, two further generations have been bred. Only once among nine broods with sufficient numbers the type with only females appeared in the first inbred generation. There are two normal broods in F_3 , otherwise females, males, and mosaics appear. From the one brood with only females, 21 individuals were crossed to males of a normal race from Maikop. None of these broods behaved as the maternal brood. Only one brood contained ♀ and ♂ Mo in the ratio 3 : 1. Five broods had females and males in a 1 : 1 and 2 : 1 ratio and in addition

a few male mosaics. In 4 broods females and male mosaics were present in what looks like 3 : 1 and 2 : 1 ratios and in addition a few normal males. All the other broods had females, males and mosaics, and here most of the ratios occur which have been enumerated. The presence of these types suggests a segregation of genes responsible for mosaic males and for lethality of others and the presence of heterozygosity for these genes in both parents.

A very different result was obtained when females from this abnormal line were crossed to males from Turkestan (Fergana; we shall include also Bochara and Samarkand in the geographical designation). Only three crosses are available, all showing only females.

2. Very different is the behavior of the second abnormal line. Here the abnormal behavior appeared in a F_1 between Poltava and Woronesch. Females from F_2 of this cross were crossed to Turkestan males. In six cases no case of only females appeared. This shows that the mothers had not the same genetical constitution as in group 1. This again rules out the maternal gene F and shows that other genes are involved, not the sex determiners. Actually these to all purposes identical crosses (if it were a question of F and M) gave five different results, namely:

	♀	♂	♂Mo	Types
$F_2 \times \text{Ferg.}$	146	1	43	♀ : ♂ 3 : 1, all ♂ Mo
do. . . .	105	--	4	only ♀, a few ♂ Mo
do. . . .	56	3	22	♀ : ♂ 2 : 1, most ♂ Mo
do. . . .	75	40	33	♀ : ♂ 1 : 1, $\frac{1}{2}$ ♂ $\frac{1}{2}$ ♂ Mo
$F_2 \times \text{Boch.}$	177	34	104	♀ : ♂ 1 : 1, 1 ♂ : 3 ♂ Mo
do. . . .	89	37	31	♀ : ♂ 1 : 1, $\frac{1}{2}$ ♂ $\frac{1}{2}$ ♂ Mo

This again shows that both parents had genes in homozygous and heterozygous condition, responsible for the abnormal phenomenon. At least two pairs of genes must be involved.

The same line inbred gave in F_1 and F_2 similar combinations. In addition to the types found in the last mentioned series, four broods, sufficiently large, with only normal sexes occur in a 1 : 1 and 3 : 1 ratio. This shows that the genes introduced by a Bochara male are probably not different from those in Woronesch males.

3. The third abnormal line, Poltawa \times Woronesch, No. 96 gives different results. Nineteen of these females from F_1 (containing 39 ♀ 5 ♂ Mo) were backcrossed to Woronesch males. Four broods had only females, 1 had a few mosaic males in addition to females, 5 (only 2 sufficiently large) had normal sexes, 3 a few mosaic males added to these, and the rest all three classes (with numbers too small for ratios). This corresponds to the behavior of the other lines, when inbred.

Females from these back-crosses were mated to males from pure races. In a first group are Odessa males. Among seventeen such crosses, broods with only females never occurred. The Odessa race, then, did not carry the gene which obviously is needed in homozygous condition to have the lethal effect upon most males. Not less than six times only females and mosaic males are found, and if we add four further broods with only a few additional normal males, we find ten such broods. This strongly suggests that the gene which has a lethal effect upon the males in homozygous condition produces the mosaic males (with a little fluctuation into normal) if heterozygous. The remaining broods have both males and mosaic males, and the following ratios occur.

- a. 1 ♀ : 1 ♂, males 3 ♂ : 1 ♂ Mo
- b. 1 ♀ : 1 ♂, males 1 ♂ : 2 ♂ Mo
- c. 1 ♀ : 1 ♂, males 2 ♂ : 1 ♂ Mo
- d. many ♀ : few males (8.4 : 1), 3 ♂ and 7 ♂ Mo.

This gives further information: (1) The mothers were not identical in regard to the gene just discussed, which shows that the maternally inherited female determiner is ruled out. (2) Besides the gene which causes mosaics when heterozygous at least another pair of genes is involved, contained in both parents in homozygous or heterozygous condition.

4. A further step may be derived from crosses of the back-cross females from this line [(Polt 96 \times Wor) \times Wor] with Turkestan males. All resulting males will be $F_{\text{Polt}} M_{\text{Wor}} M_{\text{Turk}}$. If intersexuality is involved all such crosses ought to give the same results. In fourteen such crosses to Bochara males, four gave only female offspring (one with a single mosaic male) all the others females and mosaic males, occasionally one or two extra males. This shows that one fourth of

the mothers contained an autosomal gene lethal for males, three-fourths did not have it or had it in heterozygous condition as the ratios of ♀ : ♂. Mo were either 1 : 1 or 2 : 1, 3 : 1, and many : 1. The latter ratios show further that other genes must be involved which might be heterozygous in both parents.

Another series of fourteen such crosses to males of another Turkestan race, Fergana, gave five times only females, six times females and mosaic males in different ratios, and occasionally a few males. In addition there are two crosses with many females and a few males and mosaic males each, and one brood with 3 ♀ : 1 ♂, the latter half normal half mosaic. Again another additional genic recombination must be involved which permits the development of normal males.

There are many more similar combinations available, involving as fathers hybrids between Woronesch and Turkestan. Exactly the same types of broods appear. The generation now under discussion also was crossed to Odessa males. Again, just as in the crosses with females of a former generation (but of the same F/M constitution), females, males, and mosaic males were produced in different ratios, showing that the Odessa race contains the genes needed for normal males but does not contain a gene which is needed in collaboration with some gene from the mother to produce the lethal effect upon all males.

The enumeration of these facts shows that it will hardly be possible to unravel the whole situation, as the necessary test crosses are missing. But we may arrive at an approximate solution. The following facts have to be pointed out first. As KOSMINSKY realized, the broods with only females are probably such in which all males are so extremely „intersexual” that they die. An occasional high grade mosaic testifies to this. This is a perfect parallel to the cases described by myself in real intersexuality. The lethal gene, then, is one which produces the lethal degree of mosaicism in males. Further, when males and male mosaics are present only the lower degrees of mosaicism are represented. Obviously then, different genes are concerned with the production of this type.

Let us assume that the gene responsible for the lethal effect is an autosomal dominant. Males which perish may then be LL or Ll, surviving males ll. Females from all-female cultures will therefore be

LL or Ll. Crossed to normal males ll either all Ll males are produced (only females) or $\frac{1}{2}$ Ll $\frac{1}{2}$ ll = 2 ♀ : 1 ♂. The reported facts indicate now that females from all female cultures produce (a) with Turkestan males either all female offspring or females and mosaic males. In the majority of crosses only these types appear and the latter in ratios ♀ : ♂ = 1 : 1, 2 : 1, 3 : 1, and many (ca. 8) 9:1. This shows that additional genes are involved which may allow Ll males to be medium grade mosaics, thus making a 1 : 1 ratio possible. Let us assume a second gene A which might be present homozygous or heterozygous or also absent in the different Russian races. Crosses then may be of any type in regard to this gene. We may then have the following types:

Male LLAA	= lethal
LLAa	= lethal
LLaa	= lethal
LIAA	= lethal
LIAa	= medium mosaics
Llaa	= low mosaic
llAA	= males
llAa	= males
llaa	= males

Then the following results would be possible:

1. ♀ LLAA × ♂ llAA = only ♀
2. ♀ llAA × ♂ llAA = normal sexes
3. ♀ LIAA × ♂ llAA = 2 ♀ : 1 ♂
4. ♀ LLAA × ♂ llaa = 1 ♀ : 1 ♂ Mo (without normals)
5. ♀ LLAa × ♂ llaa = 1 ♀ : 1 ♂ Mo (with eventually a few normals)
6. ♀ LLAa × ♂ llAA = 2 ♀ : 1 ♂ Mo
7. ♀ LLAa × ♂ llAa = 8 ♀ : 3 ♂ Mo
8. ♀ LIAA × ♂ llaa = 2 ♀ : 1 ♂ : 1 ♂ Mo
9. ♀ LIAa × ♂ llAA = 4 ♀ : 2 ♂ : 1 ♂ Mo
10. ♀ LIAa × ♂ llAa = 8 ♀ : 5 ♂ : 2 ♂ Mo
11. ♀ LIAa × ♂ llaa = 4 ♀ : 3 ♂ : 1 ♂ Mo

This list contains (already) the majority of the observed ratios, and it is therefore easily imaginable that a few simple assumptions would account also for the ratios not represented here. It is of no use to go into more detail. It is not my intention to prove that just this formulation is correct by applying it to all crosses. This might be

done profitably only if enough simple crosses were available. The important point is, moreover, to demonstrate (1) that the behavior of the three abnormal lines has nothing to do with the F/M balance, which accounts for typical intersexuality; (2) that it is obviously the result of the presence of a small number of mutant autosomal genes giving simple recombination results, proper combinations producing low, medium, and high-lethal types of mosaic males. The action of these genes is sex-controlled, not affecting females. (3) One of the decisive genes, namely the one called here L, is present as a mutation in the three abnormal lines. The other called here A, and perhaps a third, B, is present or absent in the different Russian strains. Turkestan, Odessa, and other strains obviously are differing in regard to the possession of these genes.

Thus it is clear that the behavior of KOSMINSKY's three abnormal broods and their offspring has nothing to do with the typical behavior of intersexuality and therefore does not offer any basis for explaining or criticizing the analysis of intersexuality.

There is, however, in the analysis of intersexuality one point which in a certain respect resembles the cases of KOSMINSKY, and this is the action of the modifier T as KOSMINSKY himself realized. The gene t is a modifying gene which acts only when homozygous tt upon males of the constitution $F_{\text{strong}} M_{\text{weak}} M_{\text{weak}}$. It is found in all weak European races but is missing in the weak Japanese Hokkaido race. F_1 between a Japanese and a European race is then Tt, and in F_2 tt is formed by recombination. The combination of tt with $F_{\text{strong}} M_{\text{weak}} M_{\text{weak}}$ gives intersexual males, which happens in $1/8$ of the F_2 males. In F_3 etc. different combinations are possible which might give normal sexes or males and intersexual males in the ratios 1 : 1, 3 : 1, 7 : 1. One rather rare combination (one in 64) will produce only intersexual sons. In this case the father must himself be an intersexual male.

It is obvious that the only similarity between these results and KOSMINSKY's lines consists in the fact of segregation* of something. Otherwise everything is different. I mention only that normal fathers of normal weak races may produce all-mosaic sons and all the other types described.

e. Kosminskys interpretation

We must finally look at KOSMINSKY's own interpretation of the behavior of his three abnormal lines. He agrees completely with the author in assuming male genes M in the X-chromosomes, though he prefers (without any proof) more than one such male genes. He assumes, further, female genes F in the autosomes and in addition female supplementary factors inherited maternally, called G. Thus far, everything is identical with my assumptions, though I called the maternal gene F and left out eventual autosomal F genes for which no proof exists. Only in my latest papers I pointed out that for the sake of unity the autosomal F genes may also be assumed. Finally, KOSMINSKY assumes the modifiers T, as also used by me. On this basis, which is completely identical with mine, in spite of assertions to the contrary, the three abnormal lines cannot be explained. (KOSMINSKY calls the differences between strong and weak genes qualitative instead of quantitative; I cannot discover any meaning in such a statement). In the three abnormal strains, according to KOSMINSKY, a mutation has occurred different from the one which occurs when a weak „G” is transformed into a strong one. This mutation acts as an inhibitor of the action of weak M genes (only of weak M, not of strong M). This is indeed an amazing statement after pages and pages of declarations of independence from my views. Translated into plain language this explanation means: Intersexual males are usually produced as GOLDSCHMIDT claimed, by the balance $F_{\text{strong}} : M_{\text{weak}} M_{\text{weak}}$. In the abnormal cases they are produced with a weak M. Therefore the F (called G by KOSMINSKY) consists at least of two parts, one of which has changed so to inhibit the action of weak M only. F is simply divided into two, in order to allow for the two types of results without any change of the general theory. Strangely enough KOSMINSKY does not even realize that he simply states the results in terms of the old theory which is stretched just a little to fit. But as a matter of fact this does not lead anywhere, even assuming that his male mosaics are typical intersexes. As we have shown, the decisive basis for such an assumption is missing in his experiments, namely a constant action of a maternal F (his G). We saw that the

different results obtained from sister females exclude an explanation upon the basis of any property of F (G), but require an explanation by autosomal recombinations.

But there remains still one difficulty which we have to face just as KOSMINSKY did, namely that the autosomal L (combinations with AA and aa) act only upon males containing two weak M. (In KOSMINSKY's terminology the mutated part of G impairs only the action of weak M.) Males with $M_{\text{strong}} M_{\text{weak}}$ bred with KOSMINSKY's lines are always normal. This is of course interesting and remarkable, but it does not mean any serious difficulty. Sex-controlled autosomal genes are known to act only in the genetic constitution of one sex. Modifying genes of different types are known to act only upon definite genetic constitutions. There is no reason why the mosaic genes in KOSMINSKY's strains should act upon all males, instead of upon only those of a definite genetic constitution, namely those with two weak M.

f. Conclusion

Thus it has been shown (1) that KOSMINSKY's three abnormal broods and their offspring are based upon an autosomal mutation, probably of two or three genes, which produce a mosaic effect upon males containing two weak M, an effect which increases proportionally to the number of dominant genes present; (2) that, even assuming these males to be intersexual, nothing has been changed in the genetic explanation of intersexuality but the addition of a new type of production of intersexes by autosomal mutations, independent of the F/M balance, which works normally. This is paralleled by LEBEDEF's *Drosophila* intersexes, as opposed to BRIDGES' triploids.

It remains to find out whether those male mosaics are identical with the typical male intersexes, or whether they represent another morphological type of sex-intergrade. The next chapter will take up this problem.

C. MORPHOLOGY OF KOSMINSKY'S MOSAICS

When KOSMINSKY's first communication about his so-called male intersexes appeared, the morphological differences from typical male intersexes seemed to me to be so large that a different phenomenon

appeared to be involved. The main point was that mosaic formations were so frequent and that low type intersexes contained female parts. I assumed therefore that a mosaic formation comparable to gynandromorphism was involved. In my own work something comparable had been found, the so-called Gifu-type of female intersexuality where intersexuality was combined with a strange mosaicism and which also was produced only when special genes of a definite race were involved. When I later thought to have found a genetic explanation for KOSMINSKY's three exceptional broods within the frame of intersexuality, I was willing to overlook those extra features because I knew from the Gifu case (and others, see later) that mosaicism may be added to intersexuality. This my position was strengthened by the fact that KOSMINSKY's series of ascending intersexes behaved normally when studied in group averages, which meant that the rules for intersexuality held, but that a special additional fact had come into play.

In the light of KOSMINSKY's complete publications this standpoint has to be revised. As the foregoing analysis shows, the genetic facts prove that KOSMINSKY's males are produced on a very different genetic basis from that typical for male intersexuality. Therefore all the morphological differences between these and typical intersexes assume a new significance and point out that a different phenomenon is involved. It is a matter of convenience whether we shall call this a different type of intersexuality or mosaicism. I prefer the latter, as intersexuality has a definite meaning. In *Drosophila*, as mentioned, three very different types of sex-intergrades are known, and all are called intersexes. All are genetically and morphologically different, only one type, the triploid intersexes, falling under the definition of intersexuality. The two others (LEBEDEFF's and STURTEVANT's) are very different, and their embryonic origin is not yet clear. Without further discussion of nomenclature we shall call KOSMINSKY's sex-intergrades, mosaic males. We have to demonstrate now (1) how they are differing from typical intersexes, (2) why they nevertheless resemble intersexes if not examined too closely, (3) which facts are known to explain their origin.

a. Morphological differences

We enumerate first the points in KOSMINSKY's description of his mosaic males which distinguish them from typical male intersexes:

1. *Antennae*. The typical male intersexes have normal antennae up to the highest grades of intersexuality. Only then smaller or larger parts of the anterior row of branches shorten and become finally completely female. In still higher grades also the posterior row becomes more or less female. These facts are based upon the study of thousands of individuals. KOSMINSKY's mosaics always show antennal changes in the lowest type of intersexuality. These are of thoroughly mosaic character. Never the whole antenna is concerned but individual branches or groups of such. Both the anterior or posterior rows may be involved. The branch may be completely split into a female and a male branch, with typical structure and color. These changes may be more extreme than in the highest grade intersexual males, though the wings and other organs are hardly changed. KOSMINSKY himself noticed, of course, this difference and tried therefore to compare these mosaic males from the three abnormal broods with real intersexes. In one case he had an F_2 from an unknown strong Japanese race with Turkestan and Woronesch, and the resulting male intersexes were even more mosaic than in the former case. But there was one difference: the anterior branches of the antennae behaved like in real intersexuality. This makes it clear what had happened: he had real intersexes plus the mosaic action introduced by the Turkestan and Woronesch races. (I repeat the fact that the only genetic exception recorded in my experiments was in a cross with Turkestan races. see p. 5) That this is correct is proven by his second combination: He combined the strong F from my race Akita with the weak M from normal Russian races (two normal races not used otherwise), and the antennae of the intersexual males were normal, exactly as in my broods. The abnormal behavior of the antennae in KOSMINSKY's mosaic males then is the result of the action of the mosaic genes contained in the respective Russian races.

2. *Gonads*. Already in the lowest type of mosaics (hardly changes in wings and other organs) gonads were formed with female color, development of egg cells and organization of egg tubules. In typical

intersexes such changes occur only when a high grade of femaleness in all other organs has been attained. For years I dissected every high grade male intersex in order to find the transformation of the ovary into a testis and only the extremest type showed the beginning of this process. In KOSMINSKY's mosaics transformations into free ovarian tubules were found, never observed in innumerable intersexes, and this in mosaics with mostly male structure.

3. *Ducts*. In individuals with little change in other organs, mosaic parts of the female ducts were found and the formation of one (or two) Bursa copulatrix on the 8th segment. Never anything comparable was observed in real intersexes, though such conspicuous structures as a Bursa, visible on the pupa shell, would have hardly been overlooked.

b. *Gradation*

These are then the differences. We must now see why this different type of sex-intergrade agrees largely with the type of genuine intersexuality.

The resemblance of KOSMINSKY's mosaics to typical intersexes covers the following points: The wing mosaic of smaller and larger patches of female color upon the male wings is in both cases alike. The copulatory organs behave practically alike, showing a transformation of the uncus into two labia, but no further changes, even in the extremest types. There is finally a definite correlation in the change of different parts. In typical intersexes first wings change, later uncus, later antennae and finally gonads. In the mosaics there is a considerable amount of irregularity. But on the other hand there is also a certain amount of regularity. In great lines namely, i.e. calculating averages, the same seriation is observed as in intersexes. With increasing femaleness of one organ all the others are increasing too. I had pointed to this before based on calculating group averages, and KOSMINSKY calculated correlation-coefficients which came up to about 0.5.

What now does the similarity as well as the dissimilarity of the two types of sex-intergrades mean? There are two possibilities: either KOSMINSKY's mosaics are typical intersexes with some additional special features, namely an inclination towards mosaicism,

or they are a special type of sex-intergrade resembling typical intersexes for definite reasons. There are analogous examples available for both possibilities: (1) We described the strange Gifu-type of female intersexuality. Here we have forms which in great lines behave like female intersexes, both genetically and morphologically. But to this is added a strange type of mosaicism, introduced genetically by definite Japanese races from the Gifu-region, probably with a gene for mosaicism. The mosaic structures are found here in all organs, in the antennae, the wings, the genital armature, etc. It is then actually possible that an irregular mosaicism is added to typical intersexuality.

(2) Different types of sex-intergrades may show such similarities that they are generally called intersexes, though they are genetically and morphologically different. In *Drosophila* three such types are known: (a) the triploid intersexes which are genetically and morphologically strictly comparable to the typical *Lymantria* intersexes. (b) LEBEDEF'FF's „intersexes", caused by a mutant gene (or two) and morphologically completely different. (c) STURTEVANT's intersexes, caused by a different gene and different in structure from both.

If we assume that KOSMINSKY's intersexes are a male parallel to the Gifu-type females, we face the difficulty of the genetic situation: their production is independent of the genic balance (namely with in a weak race). The two or three mendelizing genes, which obviously are at the basis of the phenomenon, must under this assumption, be supposed to produce first intersexuality in the typical way and second a certain amount of breakage of the correlation of the organs typical for intersexuality, in the form of mosaic formation. As a possibility this cannot be denied. If it is the correct explanation, KOSMINSKY's male intersexes are a new type of male intersexes produced independently of the genic balance and endowed with developmental irregularities in the form of mosaicism. One of the consequences of this view would be that no conclusions upon the typical phenomenon of intersexuality could be derived from this material, neither in regard to genetics nor to morphology.

There is now the second possibility. Let us assume that the genes contained in the Russian races are genes producing mosaicism. This means that they eliminate one of the sex-chromosomes during development, thus producing female parts in the male i.e. gynan-

dromorphism. Genes acting in this manner are known in *Drosophila* e.g. the claret gene and the minute case. In the first part of this paper we saw that obviously in KOSMINSKY's case different combinations of 2-3 genes have a quantitatively different effect, from low to high „intersexuality". Let us now suppose that this quantitative difference is based upon the time at which the effect takes place: aabb causes chromosome elimination late in development, AAbb earlier, AABB still earlier, etc. Late elimination means that wherever it occurs, it cannot have a visible effect except upon still undetermined organs having a not dependent type of determination. Earlier onset of the elimination means an effect upon all organs in that position at an earlier time. In other words the effect works exactly as the turning point in intersexuality. As obviously this elimination does not take place in all cells of the body (as known from the cases in *Drosophila*) the general correlation produced by the time factor is broken to a certain extent by the irregular local action, and a certain amount of mosaicism and lack of correlation is produced. There can be no doubt that this explanation is perfectly possible.

As no decisive experiments are available it is not possible to decide between these two possibilities. As a matter of fact they are not the only ones. I mention only one other one which in future might turn out to be of greatest importance: SCHULTZ, in studying the variegations found in *Drosophila*, discovered that they are due to the loss of small chromosome segments where a part of a chromosome is translocated to a heterochromatic region. This mosaicism shows some very remarkable additional features. There is a close correlation between the extent of variegation in a given tissue and the stage of development at which that tissue is laid down. With a change of the heterochromatic balance of the nucleus, whole organs are affected almost as a unit. In addition NUJDIN found a maternal effect of the heterochromatic regions upon variegation. As far as these facts may be understood from the short reports published thus far, they show some remarkable features parallel to the case under discussion. Maternal effect = F effect in *Lymantria*; somatic variegation = sexual mosaic; time of action in development = turning point in intersexuality; loss of chromosome fragment = loss of sex-genes (gynandromorphism). It is obvious that a variegation phenomenon of the type studied by SCHULTZ but involving the locus of the sex-

gene would give exactly the type of combination of intersexuality and mosaicism as found in KOSMINSKY's mosaics and in our Gifu type. It may be added that here also an explanation may be found for the strange type of wing color mosaic in small splashes which I found as well in low grade female intersexes as in the highest grade in certain combinations and regarding which I repeatedly remarked that I am at a loss to understand them.

Whatever now the explanation of KOSMINSKY's mosaics might be, one thing is sure, that they have special features not met with in typical intersexuality and which therefore cannot be used for criticizing the explanation of typical intersexuality. This leads to a further phase of KOSMINSKY's work.

D. KOSMINSKY'S MOSAIC MALES AND THE TIME LAW OF INTERSEXUALITY

The time law of intersexuality which contains the morphological explanation of typical intersexuality reads: An intersex is an individual which begins development with its gametic sex and ends it, from a turning point on, with the other sex. The grade of intersexuality is determined by the time of incidence of the turning point. This law had been derived from innumerable consistent facts. On the basis of the study of his mosaics KOSMINSKY declares it to be invalid.

a. *Kosminsky's own interpretation*

Let us first see what he puts in its stead. Of course KOSMINSKY cannot deny that intersexual females begin development as females and intersexual males as males, and he calls this an undeniable fact. Therefore he can also not deny the existence of the turning point. But this turning point is supposed to occur always at the same time early in development. But, of course, there is even in his own mosaic material the general positive correlation between the amount of intersexual change in all organs, which is so well explained by the time law. As this is to be refuted, something else has to be introduced to explain the regularity. This *deus ex machina* are the modifying genes. Their action begins later than the action of the basic genes and their action produces an upset of the genic balance. The amount of

this upsetting is typical for the different modifiers, some having a strong action thus producing high grade intersexuality, some acting less strongly and producing less intersexuality. This does not suffice yet to explain the orderliness of the intersexual changes. Therefore it is assumed that the different organs have a different degree of stability and therefore respond differently to the different degrees of modifier action. Finally some of the organs react to these modifiers by intermediate development and others with an all or none effect i.e. change of sexual differentiation.

Looking at this interpretation and comparing it with the time law of intersexuality I must frankly say that I should prefer to stick to the time law to the last straw if there were only the faintest hope of proving it, before resigning to the acceptance of such a product of embarrassment.

b. *The time law and the facts of development*

Is there now actually anything in KOSMINSKY's work which raises difficulties for the time law of intersexuality? Here are his arguments: The time law was based on the discovery that the latest organs to be differentiated are the first to be changed in intersexuality and vice versa. According to KOSMINSKY the facts do not agree with this assumption. These are his objections:

1. The saccus in front of the ring of the male genital armature differentiates very late; the same is true for this ring-like segment. Nevertheless they change only in the highest grades of intersexuality. Here we have a characteristic type of erroneous argument. Ring and saccus are parts which have no equivalent in the female. Once their development is started it cannot be changed in a female direction because no such exists. Either the further development can be stopped or it will be finished. These two organs are actually chitinized at the end of development. But their organization has been started much earlier as their configuration depends upon the special arrangement of the last segments which is differentiated prior to pupation. This once determined, it will follow its course or stop further differentiation. Actually it does the first and only in high grade intersexes its differentiation is impaired, namely because the turning point arrived already when the segmental arrangement was

laid down. The behavior of this segment in male intersexuality then agrees completely with the time law. I may add that the behavior of the same segment in female intersexes is another beautiful demonstration of the time law, a set of facts which KOSMINSKY is careful not to mention.

2. Sex cells differentiate very early and therefore ought to change only in higher intersexes. KOSMINSKY's mosaics showed eggs in the testes in low grade intersexes. This is another example of wrong reasoning and disregard of the facts. Both female and male intersexes show a typical series of transformations exactly parallel to time of beginning of change (of the turning point). In typical male intersexes transformation of sex cells occurs only in the highest grades. The reason is that in the normal testis spermatogenesis is already finished in the older caterpillar and therefore no material available which could change into female growth. If, however, in a testis with late turning point some spermatogonia would be left, they could still grow as ovocytes. This happens actually occasionally and is in full accord with the time law. A different thing is the testis transformation in some of KOSMINSKY's mosaics. This is an unusual behavior showing the peculiar nature of these mosaics from abnormal broods.

In connection with this argument KOSMINSKY also mentions that in high grade female intersexes still ovarian cells are found, which ought not to be possible after an early turning point. It is almost embarrassing to answer this criticism. Growing ovocytes and nurse cells may be destroyed after the turning point (phagocytosis!) or they may remain. But they cannot be transformed into spermatogonia. Actually they are partially destroyed and in part they survive, especially the nurse cells, as described in detail in my papers. How this should reflect upon the turning point and time law is beyond my understanding.

3. In the sex ducts of male intersexes the first female transformation occurs in the part called „stalk of the egg tubules". The vas deferens is not affected yet, though it differentiates later. Again we have the same story as before: The stalk exists in both sexes and may be transformed from a male condition into the female one by growth when the turning point arrives. But the vas deferens is not homologous to the female ducts and therefore does not come into play at all. Here again it must be added that the series of female

intersexes behaves in regard to the stalk exactly as demanded by the time law.

4. KOSMINSKY claims that no organ changes completely to the other sex, as the time law would require, with the exception perhaps of antennae and uncus. This is a strange statement. A complete change of course requires an early turning point in order to have sufficient time for this change. Actually this complete change is found wherever it is possible, namely: (*a*) male and female antennae; (*b*) female to male color (the splashed, mosaic, highest grade intersexes are a special case) in all female intersexes; (*c*) male to female color in a very few surviving specimens; (*d*) wing scales and frenulum in both directions; (*e*) wing size and shape in both directions; (*f*) shape, size, color, hair of abdomen in both directions; (*g*) uncus in labia and vice versa; (*h*) the male segmental ring and saccus in female intersexes; (*i*) penis and valvae in female intersexes; (*k*) oögenesis into spermatogenesis and vice versa; (*l*) ovarian tubules into testis compartments and vice versa; (*m*) testis stalks into oviducts and vice versa; (*n*) vas deferens and glands in female intersexes. In other words the complete transformation occurs in every single organ, sometimes in earlier grades, sometimes only rarely. Among the many ill-founded arguments of KOSMINSKY this is the most incomprehensible one.

5. KOSMINSKY emphasizes the individual differences found in regard to the correlation of changing organs. His main contentions are derived from his abnormal males with relatively frequent mosaic parts. We discussed this matter fully already, but still one point ought to be brought out. In such parts of his papers in which KOSMINSKY tries to refute the time law this abnormal mosaic behavior of the males in his abnormal lines is the decisive feature: no correlation of intersexual changes in different organs as the time law requires (and as it is typical for real intersexes). In those chapters, however, in which he wants to prove that his mosaics are typical intersexes again the good correlation is emphasized as most important, and high positive coefficients of correlation are calculated. Thus the critic himself demonstrates the valuelessness of his argument.

6. The time law requires that differentiation is first in the direction of the gametic sex and then switches over to the other sex. The first part is not denied by KOSMINSKY, who thus without noticing it,

accepts the decisive point of the time law; but he claims that after the turning point (a conception which he simultaneously accepts and denies) development does not take place with the other sex but is intermediate. This claim is certainly disproven at the outset by the fact that in all organs all stages of development towards the other sex up to complete normal appearance are found. It is also completely refuted by the facts regarding the development of intersexual gonads and genital armature as found by myself and my collaborators. KOSMINSKY himself has studied only the uncus in male intersexes and antennae in one type of female intersexes. It is most illuminating what he tells about the development of the uncus in male intersexes. His description shows that it happens exactly as the time law requires and he adds that one might indeed describe it this way. But then he bluntly states that this development is not female after the turning point but intermediate, thus annulling his own description by a perfectly gratuitous statement. As a matter of fact the transformation of the uncus into labia in male intersexes is very typical for the time law. On the basis of comparative morphology, the uncus is homologous to the dorsal part of the labia, the spread of which ventrally means a leaflike outgrowth from the original anlage. The uncus concresces from a paired anlage which moves dorsally for that purpose, as KOSMINSKY first described. The seriation of development then is: paired anlage of dorsal uncus, concrescence. In male intersexes first the concrescence is stopped sooner or later, giving a bifid uncus, and then development of the labial spread begins. If this begins early enough (high grade intersexuality) a perfectly normal pair of labia is formed. If it begins a little later the development of the dorsal ridge into an uncus has already started. No concrescence occurs, but while the ventral spread of the labia is formed the already initiated growth of a pointed dorsal structure continues, and the result is a pair of atypical labia with a rudimentary pair of unci attached dorsally, as pictured in my papers.

7. The only other organ the development of which has been studied by KOSMINSKY are the low grade intersexual antennae of females. He repeats here only his former description in which he claims that these antennae differentiate as intermediates from the beginning. In a former paper (U. Int. IV p. 236) I have shown that this apparently intermediate development is in fact first female and then male if

analyzed in terms of different component processes. As both sides have nothing new to add to this discussion I refer to the older publication.

8. A special chapter are the antennae of intersexual males. They remain male up to the highest degrees of intersexuality, when first only the anterior row is becoming female, later also the posterior one. I had shown that the posterior row differentiates first in development and in a different way from the anterior one, which was confirmed by KOSMINSKY. The male type of differentiation of the antennal disc must be determined very early in development, as the two types are already completely prepared at the time of pupation. The behavior of the male intersexes is then in perfect harmony with the time law. But KOSMINSKY declares that the constancy of male antennae up to high grades of intersexuality in typical intersexes does not agree with the time law. I cannot understand this argument. Of course KOSMINSKY's mosaic males show also the strange mosaic behavior of individual antennal branches in low grade intersexes. But this is the special feature of these mosaics, which differentiates them sharply from real intersexes.

9. KOSMINSKY's last argument applies to the wings of intersexual males which still in high grade intersexes have streaks of male colours left and which in the lower grades already show their typical condition soon after pupation. (KOSMINSKY's mosaics show the mosaic wing already indicated upon the pupal shield. I never observed this but as it is possible that I overlooked this, I shall not stress this point). I have frequently pointed out myself that the behavior of the wings in intersexual males offers a very difficult problem. In typical female intersexes male wing color appears as a whole in low intersexuality. This shows that after a late turning point pigment may still be formed and deposited in the scales. But in intersexual males (also in Gifu type and splashed mosaic females) the female color appears in spots of increasing size with increasing intersexuality, and these spots are also structurally female and are certainly determined around pupation time or earlier. This shows that the determining mechanism in both sexes must be different. Now we know that the time elements of male and female differentiation in *Lymantria* are very different: The male caterpillar grows faster, but has a larger pupal rest. Male gonads are fully differentiated

(sperm!) in older caterpillars; ovaries develop mostly in the pupal stage. With this corresponds exactly the behavior of intersexes: female intersexes of lower grade may have ovaries of the pupal type (plus later male changes); whereas male typical intersexes of medium grade still contain testes. If then wing determination in regard to the male or female pigmentation and form of scales follows the differentiation of the gonads, it will occur earlier in the male. Now we know from experimental evidence in Lepidoptera and Diptera that the general architectural features of the wing are determined very early in the imaginal disc and that only special features in patterning and pigmentations may still be influenced up to soon after pupation. It seems that also the type of general pigmentation is included in this early determination of form, growth, and venation in the male of *Lymantria*, but that this determination is not finished until after pupation. We have discussed the process of this slow determination by a determination stream at other occasions.

I repeat again that the behavior of the wings of male intersexes presents a very difficult problem. Though the known facts are best described in terms of the time law, I agree that some facts are still unknown which are needed to make the interpretation a certainty. I never have tried to hide this difficulty, which may be overcome only by more experimental work upon the imaginal wing-disc.

It has now been shown that KOSMINSKY's criticism of the time law of intersexuality has completely failed. His own conception of a single turning point, at one time only for all grades of intersexuality, with a consequent intermediate or intersexual development, is neither substantiated by the facts nor inherently probable.

E. BALTZER, BONELLIA AND THE TIME LAW OF INTERSEXUALITY

In a series of recent papers BALTZER has entered the field of discussion of intersexuality in *Lymantria*. In his classic work on sex-determination in *Bonellia* BALTZER had found a long time ago that larvae which developed freely became females, larvae attached to a female's proboscis became males, and larvae, attached for some

time and removed again, formed sex-intergrades. He assumed that the latter had differentiated as males first and finished as females after removal from the proboscis, thus stating first for *Bonellia* a parallel conception to the time law which we found soon afterwards in *Lymantria*. Later however BALTZER found facts which made him change his view and give a different explanation for the sex-intergrades of *Bonellia*. Ever since he seems to have had the wish to explain also the *Lymantria* intersexes in terms of *Bonellia*, which requires disproving first our interpretation by the time law of intersexuality. His recent papers are devoted to this task.

If we leave out of account such discussions which are not directly concerned with the topic of this paper (discussions on the theory of heredity, for example) his material may be arranged in three groups. First, he makes the maximum use of KOSMINSKY's material. Second, he tries to disprove my conclusions on the basis of my own material; and third, he tries to construct a parallel between *Bonellia* and *Lymantria*.

We shall understand BALTZER's argumentation more easily if we report first his conclusions, derived from the studies of the *Bonellia* intersexes. Here he finds a number of processes which occur simultaneously to produce the sexual mosaics, which then are not the product of a switch-over in sexual determination but of simultaneous intersexual differentiation, which is more extreme here, less there, depending upon the reactivity of the tissue to the proboscis stimulus. The elements of this intersexual development are intermediate velocity of development, intermediate effect of a developmental process and similar things. We do not question here the correctness of BALTZER's analysis of the *Bonellia* intersexes, a problem to which we shall return below. The point is exclusively whether the *Lymantria* intersexes can be explained rather on the basis of what BALTZER considers the proper explanation of the *Bonellia* intersexes or on the basis of the time law of intersexuality. As BALTZER observes correctly, this is completely independent of the genetic side of the case but a pure problem of developmental mechanics.

BALTZER then assumes that the action of the unbalanced sex-genes which produces intersexes is not a consecutive one but a simultaneous one, i.e. an intersexual one. (He thinks this to be in agreement with

BRIDGES' type of the theory of genic balance of sex-genes. He overlooks however that DOBZHANSKY and BRIDGES have later demonstrated the time law of intersexuality to apply to the triploid *Drosophila*-intersexes). He agrees that there are certain facts which prove that the development begins with the gametic sex, even that there is some partial truth in the time law in so far as the primary development with one sex slowly glides into an intersexual development. This would mean that the genic balance is first in favor of the genetic sex and slowly glides into another equilibrium, namely an intersexual one. He does not mention that this does not yet explain the definite degree of intersexuality in different genetic combinations. The additional hypothesis would be necessary, that the time of the gliding over process depends upon the amount of the genic balance or that different intersexual equilibria are being reached finally or a similar hypothesis. (Of course thus the difference between the time law and the new hypothesis would become very small indeed). BALTZER's idea then is somewhat midway between mine and the one reported for KOSMINSKY. His opposition is against the idea that the turning point changes female into male development or vice versa. He takes the turning point as a more or less protracted affair and the result as the start of intersexual development.

To prove this viewpoint BALTZER begins by contrasting *Bonellia* and *Lymantria* intersexes. He mentions four points of parallelism:

1. In both cases intersexes have a mosaic character.
2. The individual traits in both cases may be purely female or male, or they may look like developmental stages of such. (According to BALTZER they are not such but products of inhibition). Also intermediate organs may exist.
3. In both cases different grades of intersexuality exist. But the different organs show no correlation in this respect. To carry through this parallel BALTZER must rely on KOSMINSKY's mosaics for which we already showed that they are different from typical intersexes.
4. The intersexual organs have an intersexual development from the very beginning. Again KOSMINSKY is the witness hereof for *Lymantria*. But we showed that KOSMINSKY studied only two organs, uncus in male intersexuality and antennae in female. For the former organ he reluctantly acknowledges that development looks as go-

verned by the time law but nevertheless must be called intersexual. In the case of the antennae my own work leads to the opposite interpretation from KOSMINSKY's. And for all other organs studied by myself and my students (gonads, ducts, armature) it is established that no such a thing as intersexual development exists.

Thus at the outset it looks very doubtful whether *Bonellia* may be used to explain *Lymantria*. BALTZER then proceeds to enter into an analysis of the details. He begins with a discussion of the relation of determination and differentiation, with which I agree. As a matter of fact, I kept in mind these facts of experimental embryology in my own discussions wherever the material required it. A next chapter discusses the duration of the turning point. He argues that there is not much difference between the action of a turning point and the simultaneous action of male and female genes, if there is a transitional period between the two main periods prior and after the turning point. In this connection the development of intermediate types of organs would be decisive. According to my interpretation, they are the result of differentiation over a certain period with first one, later the other sex (which ought simply to mean an inhibition of further growth). In BALTZER's interpretation such development would again have to be intermediate from the beginning. The crown witness is again KOSMINSKY with his description of antennal development in intersexes which does not agree with mine. But the discussion of these more general points may be stopped here, because their value stands and falls with the detailed facts, the question whether the details of intersexual organization and development in *Lymantria* agree with the time law or not. BALTZER himself discusses these details extensively to demonstrate the failure of the time law. We shall prove that he is mistaken.

The first organ system to be discussed is the genital armature of the intersexes, which I always considered to be the most beautiful example of the time law. Here also very complete information is available. (a) The whole series of transformations from the female into the male armature is known from innumerable specimens. (b) The reciprocal series from male to female is complete as far as it goes. (c) The development of the normal organs is known from the work of KOSMINSKY, DU BOIS and myself. (d) The development of some types of female intersexes is known through DuBois. (e) Each

intersexual individual contains definite information about one part of the development: The condition at the time of pupation is known from the sculpture of the pupal shell.

How is it now possible that facts which appear to me as most beautiful examples of the time law are considered by BALTZER not to agree with it? It is easy to show up the misunderstandings upon which BALTZER's criticism is based:

Argument 1. In young intersexual caterpillars DuBois found occasionally both the male Herold's organ and anterior female imaginal discs present. I do not quite understand this argument. The anterior pair of imaginal discs has no homologon in the male and may remain after the turning point without further differentiation. The posterior pair (9th segment) is possibly (not certainly) homologous to Herold's organ and forms such after the turning point. DuBois fig. 36, which is also mentioned in this connection, represents a Gifu type intersex which is characterized by mosaic formations upon a different genetic basis.

Argument 2. This seems to be regarded as the most potent one, as the next argument is introduced as less potent. BALTZER points out that female intersexes may have a male segmental ring together with labia and apophyses. As the differentiation of the ring occurs after the differentiation of labia and before the apophyses, the order is not the expected one. There are two serious misunderstandings in this argument. First, that the third day in a male pupa is the same as the third day in a female. As male pupae have a much slower development such a comparison of absolute times is impossible. But, second, actually the whole situation is different. It is true that the final configuration of the male ring is formed in the pupa, namely when the tip of the abdomen is telescoped into the anterior segments. The edge of the 9th segment is then chitinized as a ring. But the configuration of the last segments, which finally makes the ring possible, is already perfected before pupation. At the time of pupation the last segments of males and females have a perfectly different arrangement, and this is such that only the male arrangement permits the later formation of a typical ring. Up to high grade female intersexes the female type of segment arrangement persists to the time of pupation, as the pupal shell shows. No typical male ring may be formed when a later turning point occurs, and it

does not. But in females only a small spot of this segment, the basis of the apophysis, is later chitinized. In lower grade intersexes this chitinization increases, which is a male trait, and spreads in the series slowly from the base of the apophyses dorsally until finally it unites dorsally and ventrally into a ring. This ring is now the chitinized edge of the 9th segment but in an absolutely female configuration, size, proportions, and details of structure being very different from a male ring. And only in the high grade intersexes, which show already in the pupal case a change in structure towards the male, a male type of ring is developed. The conclusion of BALTZER that here both sexual tendencies must have acted together is absolutely erroneous, and the facts in all their aspects agree completely with the expectations from the time law.

Argument 3, introduced as less potent but sufficient to raise difficulties for the time law is the following set of facts: Apophyses may still be present when the beginning of ring formation occurs. In fact they are rudimentary in this case and always abnormal. In addition the formation of the ring is not involved in this case but superior chitinization at the basis of the apophyses as represented before. Further, in a series of female intersexes the anal cone, which is homologous to the valvae, is being reduced in beginning intersexuality in higher degrees more and more and finally slowly developed into valvae-like organs. BALTZER claims that the anal cone is a product of early development. This however is true only of the anlagen. The formation of this organ on both sides of the genital opening is perfected only very late, together with the differentiation of the labia. A later turning point will stop this differentiation and one occurring earlier and earlier will reduce the organ to the type of the anlagen plus subsequent male (valvae) differentiation, when occurring early enough. Actually this series of changes is a perfect illustration of the workings of the time law. BALTZER was deceived into this argument by taking the early anlage as typical without thinking of the late actual differentiation.

There is still another example, from which BALTZER would derive an actual inversion of the time law. In some female intersexes in which labia were not yet transformed into uncus, the anlage of a Herold's organ had been described in 1919. If this would mean an early formation of a Herold's organ before the turning point, it

would not agree with the time law. But of course the claim was not this but a new development of Herold's organ after a late turning point was supposed to take place. But as a matter of fact the whole discussion is futile, because I later found that the structure which I had taken in 1919 for the primordia of Herold's organ were chitinizations within the sex-ducts (see 1927), and that valvae and penis are formed differently in intersexes (from the anlagen of the anal cone in the lower grades and only in the higher grades from a real Herold's organ). BALTZER mentions in this connection also cases in which the uncus is not completely fused in medium grade intersexes. I do not understand this point as this is exactly according to expectations.

Argument 4 has to do with the development of Herold's organ in late stages of intersexes which BALTZER does not think to be possible. I agree with him and add that this assumption was based on a misinterpretation which however had been corrected long since, as just mentioned (see 1927, p. 78-79).

Argument 5 points to the fact that in male intersexuality the segmental ring of the 9th segment remains unchanged to almost the highest observed degrees of intersexuality and changes only then. The fallacy of this argument has already been exposed in the second part of this paper: (1) The ring is determined by the configuration of the last segments which is finished before pupation. The ring then is an early not a late organ. (2) Chitinization, which is the late process, chitinizes what is present. (3) There is no female equivalent to the ring existing into which a male segmental arrangement could be changed at a late date.

A critical review of BALTZER's arguments regarding the genital armature then shows that the facts which he considers to be in disfavor of the time law are all in complete harmony with it if misunderstandings and erroneous statements are cleared away. Actually BALTZER's criticism of this decisive set of facts has completely failed. As he certainly has combed the data very thoroughly in order to find weak points, this shows that the extensive data on the genital armature, comparative, morphological, embryological are still today, as they always were, the most perfect and consistent illustration of the time law of intersexuality.

A second chapter treats the gonads, though rather shortly. The reason is obvious. The behavior of the gonads in intersexual males and females both in morphology and development is in such perfect harmony with the time law — by the way also the ducts — that it is very difficult to find a point of attack. Here actually the complete differentiation, first with one sex and then with the other, is known for female intersexes and easily computed for male intersexes. Thus BALTZER finds only one actual difficulty, namely the frequent irregularity, insofar as the individual egg tubes and testis compartments do not always reach the same stage of transformation. I cannot see what this independence of the individual tubules has to do with the time law. A testis compartment may transform into an ovary if the stimulus from the inductive tissue is sufficient and if enough spermatogonia capable of growth are still left. Minor differences in nutritional conditions may add to such differences which are simply local accidents of development. As often as not, all tubules (compartments) behave alike, sometimes one lags, or all of one side are lagging, etc. But this is a feature which cannot be connected in any way with the time law: in all cases are all tubules of a female intersex (reciprocally in a male intersex) first purely female. In all cases a male phase follows the female one, though an individual tubule may be left behind degenerating, not being able for this or that reason to carry out the stages of male differentiation.

A last chapter finally deals with the correlation of the intersexual organs. BALTZER regrets that I did not give tables for the different organs in one individual. This is indeed regrettable. I did not compute such tables for the simple reason that everything was correlated just as I described, and the variations (which we mentioned) were so small that such tables would not show anything. One such set of dissections for which I published the data are mentioned by BALTZER. He agrees that indeed here the perfect correlation is found as required by the turning point, with the only exception of the unchanged (hardly changed) antennae (male intersexes). This case of the male antennae has been taken up already in the first part and has been shown to be according to expectation. The only difficulty to which BALTZER also points is the wing mosaic. We discussed this before.

In a second part of this chapter KOSMINSKY's mosaic males are discussed in the same sense as KOSMINSKY did. The lack of correlation

i.e. the extreme mosaic character in some individuals is accepted as a normal feature of male intersexuality, which it is not. We have shown before that KOSMINSKY's mosaics are different from typical intersexes in this respect. This rare type, derived from three genetically abnormal broods, can certainly not be used to explain the otherwise perfectly consistent normal type, as already discussed in detail.

A careful analysis of BALTZER's objections then shows that he has completely failed in trying to disprove the time law of intersexuality. Much could be said against the theory which he wants to substitute. But this is not important as long as the facts are completely in favor of the older view. I am certainly ready to change my view when the facts require it. This moment has not come however. It may be that BALTZER's explanation of the *Bonellia* intersexes is correct. In this case they represent a phenomenon which is thoroughly different from the one in *Lymantria*. We may call this also intersexuality, if we realize that it is different in expression as well as in causation from the *Lymantria* type of intersexuality.

I wish to add finally that I agree completely with BALTZER in the idea that the final word regarding all the details of intersexuality in *Lymantria* (see especially wing pattern) can only be spoken when experimental embryology will have furnished us with more insight into the details of the determinative processes in *Lepidoptera*.

F. ADDENDA

a. *Seiler's work*

This paper had been already sent to press, when also SEILER, with his students, entered the discussion on the basis of a renewed study of the triploid intersexes in *Solenobia* (Psychid moth). In his first paper (1937) he discusses the behavior of the intersexual gonad. He finds that the facts are in complete harmony with the time law, first development with one sex and after the turning point continuing with the other sex. He then asks himself whether the turning point is a short moment or not, a question which BALTZER also put. I personally cannot see the importance of this question. I always described diagrammatically the turning point as one moment. But of course

I never assumed that it actually occurs like lightning. As the general idea was that at this point the sex determining substances of the other sex gain the upper hand it is obvious that the reaction to this new situation will take some time, depending upon threshold conditions as well as the ability of a developing organ to change its course. I spoke occasionally of compromise results of the old type of growth and the new one after the turning point. I cannot quite understand why BALTZER and SEILER consider this detail as so important. In addition it seems that SEILER's argument in favor of a slow transition is very unconvincing. He emphasizes the details of a gonad which is still female near the ducts, male at the apex and more or less disorganized female in between. As I described for the identical situation in *Lymantria*, this means that after the turning point the highly differentiated distal parts of the egg tubules resist longer to disintegration than the younger parts which lose their organization and fall apart into cell groups. I cannot see what this phenomenon shall have to do with the turning point. The slow disintegration and resorption of the ovarian tube in the direction from the young (proximal) to the old (distal) part of the tube is what is to be expected. To take it as a sign of a long and slowly working phase of sex-reversal is an impossible conclusion. SEILER also emphasizes that the individual tubules of the ovary may show different stages of sex reversal, just as in *Lymantria*. Again he concludes that the turning point therefore does not occur simultaneously in all tubules. I have discussed this point above and have shown that this is a special and very irregular feature of development, which cannot be attributed to different local turning points but to different local conditions in the gonad which is hit as a whole by the process of sex-reversal but may show local differences in reactivity. Thus I am unable to find in SEILER's paper a single fact — the facts are practically identical with those in *Lymantria* — which could be used as a real argument against the old form of the turning point concept. (SEILER, of course, disagrees only with a short and simultaneous turning point.)

SEILER's work has been extended by his students, BEYER (1937) and NÜESCH (1937), who studied other organs of the *Solenobia* triploid intersexes. BEYER studied the sex-ducts. He described seven classes of intersexes from pure female to pure male type. The first

classes have only female ducts, the last ones only male ducts, as expected. The decisive classes are the intermediate ones, III and IV. Here all female parts are said to be present, though their position is of the larval type, which means a common opening for the derivatives of the imaginal disks of the 8th and 9th segment. As there is no male homologon for the disks of the 8th segment it is not surprising that its derivatives continue development after the turning point, though it is stopped short in a larval condition. Similar cases have been discussed before for *Lymantria*. Unfortunately we do not know whether the disks of the 9th segment are homologa of the male HEROLD's organ or not. In these intersexes this organ and its derivatives begin to be formed more or less, which again agrees with expectation on the basis of the turning point. Here now the question of homology comes up. If the disks of the 9th segment are homologous to HEROLD's organ in the male, no vagina and cement glands ought to exist. BEYER claims their existence, but his figure shows that what he calls „larval arrangement" is actually the absence of the part of the oviduct (vagina) which is derived from the 9th segment disks. He pictures, however, a rudimentary "cement gland". But for this one point (the origin of these glands is not so perfectly clear) his data agree completely with the turning point concept and also with the *Lymantria* data.

(In case that HEROLD's organ would not be homologous to the 9th segment disks in the female, which is not yet known for certain, the data of BEYER would exactly fit expectations). BEYER draws the conclusion from his data that the facts do not agree with the time law. The opposite conclusion would be more consistent with the data.

There is a note in this paper that the proximal parts of the ducts contain patches of epithelium of the other sex. I reserve judgment of this point until pictures of this condition will be available. BEYER finally compares the correlations between the behavior of sex glands and ducts and finds them to agree with the time law. (The facts are the same as in *Lymantria*). Some data regarding the third leg do not seem very important, as well to the author as to myself. Thus BEYER's work, as far as it can be analyzed, agrees with the time law of intersexuality, but for one small and not yet clear item, the cement gland. His different conclusions appear completely unwarranted.

NÜESCH has studied the behavior of the genital armature, eyes and

antennae. The armature shows a series of intersexual conditions which closely parallel the conditions found in *Lymantria*. They demonstrate perfectly indeed the development with a turning point. The author does not say so, but overlooking this agreement he makes the most of such minor variations as are occasionally found here as in *Lymantria*. He seems to argue that if the results of such a developmental reversal, the turning point, do not agree diagrammatically in every individual case and detail with expectation the whole law must be wrong. I think that this is bad argumentation. Minor variations within an otherwise perfect order have to be accounted for; but it is more probable that they find their explanation within the general order than that they will upset completely a well established order. No physicist, by the way, would ever use such a type of mistaken argumentation.

NÜESCH studied also the correlation between the order of intersexual change in eyes and antennae as compared to that of the armature and he found them in agreement. No agreement was found in regard to segmentation and hair formation within antennae under the assumption that the later visibility of hairs and scales in development of the antennae mean also later determination. But nothing is known about this, nothing also about the possibility of inductive determination.

Looking then at the material presented by SEILER and his students for *Solenobia* we come to the conclusion that the mass of it, if properly analysed, agrees with the time law of intersexuality. Such minor discrepancies, as seem to exist, will find their explanation, just as similar variations in normal development do. SEILER's scepticism, as it seems, largely produced under the influence of BALTZER's erroneous interpretations, appears to me therefore not well founded.

b. *The Bonellia Intersexes*

BALTZER's opposition to the time law of intersexuality is based upon his work with intersexes in *Bonellia*. It is general knowledge that in this worm indifferent larvae are developed from eggs and that these develop mostly into females (ca. 95%) if left alone. If, however, the indifferent larvae are attached to the proboscis of an adult female, they develop within a short time into the minute males which

later will parasitize the females. The male determining substance secreted by the proboscis may be replaced by extracts or even by K, Mg ions (HERBST). Already in BALTZER's first paper he described intersexes which were obtained when larvae, which normally are attached for four days to the female proboscis, are taken off after a few hours of attachment and are allowed to develop independently. BALTZER's original interpretation was that in this case a phase of male differentiation was followed by a female phase which is the same assumption as contained in the time law derived from the *Lymantria* case. Later on, BALTZER became sceptical when he found certain facts concerning the seriation in male development, and in a series of recent papers by himself and his students, ZURBUCHEN, LOOSLI, GLAUS, NOWINSKY, he tries to prove that these intersexes have a very different origin. This led him then to criticize also the time law in other cases.

Before we oppose the two explanations, that by the time law and that derived at by BALTZER, we must make clear the special features of the *Bonellia* case which have to be kept in mind. If an intersex develops in *Lymantria*, development begins with the gametic sex and continues after a certain time, the turning point with the other sex (or with intersexual development, if BALTZER were right). The incidence of this turning point is determined genetically. If an intersex of the Freemartin type in vertebrates is formed, development begins with the gametic sex; the turning point is identical with the infusion of the opposite sex hormones, which then determine further development with the opposite sex. In *Bonellia* however development is first indifferent and continues female if left alone. If, then, „hormones” of the proboscis act, development is shifted towards maleness. The attached larva therefore is in a phase which corresponds to the beginning of differentiation of a normal gametic male in the other cases. If this larva is detached from the proboscis, this moment corresponds to the turning point. After this turning point however there is neither the control of further development by a special genetic situations, as in *Lymantria*, nor a control by hormones determining the opposite sex as in the Freemartin type but a return to the natural trend of female development of the unattached larvae, provided that this is still possible. These statements describe the actual facts without any interpretation, and they must be kept in

mind if interpretations are sought. There is one further fact, discovered by BALTZER, which belongs to the special features of the *Bonellia* case and which has to be kept in mind: male differentiation under the influence of the proboscis begins at the point of attachment, the anterior ventral surface of the larva, and continues backward. (Strangely enough this seriation is said to be true also for non-attached larvae, masculinized by extracts or ions.)

Let us now define the two opposed interpretations of the origin of the *Bonellia* intersexes. According to the time law, if sensibly applied to the special features of the case, a *Bonellia* intersex produced by too early detachment from the proboscis (or by insufficient action of the ions) must have been supplied with an insufficient amount of male hormone (if we use this convenient term just for description's sake). A complete male development does therefore not succeed. When the action of the insufficient supply of hormone is exhausted, the actual turning point arrives, and development continues female. For such parts which had already reacted with this hormone. Those organs which had not yet reacted with the male hormone will simply continue female development. (It is to be noted that BALTZER has derived postulates for the time law, which he wants to disprove, which have been schematically taken from the *Lymantria* case. This might have been possible before much was known of *Bonellia*, but if the case is discussed today the assumptions have to be based on today's knowledge. On this basis the foregoing consequences follow from the time law.)

The interpretation however which BALTZER derives from his studies is the following: At the time, the larvae are detached prematurely from the proboscis they are still indifferent, at least in most organs. From this moment on, male and female determining substances act simultaneously upon development. The effect depends upon the amount of each which an organ needs for sexual determination, upon the reactivity of the organ, its state and time of determination and similar factors. From this follows a simultaneous development of male and female parts, an intersexual development into a mosaic, in which no time element is involved.

Let us now look at the facts. We leave out of account such facts as the spontaneous development of some males without attachment to the proboscis; of some pure females after attachment; further, the

variability of the effect of short attachment; and the behavior of males produced after short attachment which slowly reach a condition of complete maleness. These points, which are of interest for the general interpretation of sex determination in *Bonellia*, are not important for the problem of the time law, though BALTZER discusses both simultaneously. The problem alone involved in this paper is: time law or simultaneous intersexual development.

BALTZER describes three main types of intersexes: The first consists of individuals which are almost complete males (sometimes not fully differentiated) with only the addition of female setae. These setae are a purely female trait, which in normal female larvae is formed relatively lately. According to the turning point interpretation it is expected that in a larva which has received sufficient male „hormone“ for complete or almost complete male differentiation, the only female organ which can still be formed, if a female phase begins after premature detachment, are the setae. (Partly also the proctodaeum) BALTZER, strangely enough, concludes that the appearance of these setae proves a differential reactivity of different parts to the male hormone. He forgets that this is an organ without a sexual alternative and that therefore its actual behavior is just what the time law requires. In comparison with *Lymantria*, such an animal is a low grade male intersex, though of course it is not a genetic male.

The second main group of intersexes is more complicated. Male parts are the not completely developed seminal tube and the male body form. Female is the rectum (proctodaeum), more or less developed, and anal vesicles, also more or less rudimentary, further setae. In explanation of these structures it must be said that the seminal tube of the male is a homologon of the female's stomodaeum and that the first indifferent organ which is stimulated to male differentiation after attachment of the larva is this „Anlage“ which has an alternative of sexual development. (Here belongs also skin and body form). The proctodaeum is missing in the male and grows out from the intestine when the indifferent larva grows into the female. In normal development then the male hormone inhibits this. Again the structure of these intersexes is exactly what is expected under the time law. The male „hormone“ has succeeded in starting the development of a seminal tube and in inhibiting proctodaeal development when the larva is returned to female differentiation. The seminal tube

develops as far as the insufficient stimulus permits. Once the alternative is decided it cannot be retraced any more. But when the inhibition of the proctodaeum ceases this may grow out in the female fashion, may be with some restraint due to the primary inhibition. Setae behave as discussed before. BALTZER's explanation is very different. To him the structure is an intersexual mosaic due to an intersexual stimulus spreading from the front backward and hitting parts of differential reactivity.

The third type of intersexes has no male organs whatsoever, and the only abnormality is the inhibition or suppression of the female stomodaeum. According to the time law these are high grade male intersexes i.e. individuals which just had been pushed in the male direction: the stomodaeal growth was already inhibited but the seminal tube not yet started when the female phase set in and complete female development resulted but for the primary inhibition. I cannot see how this type could possibly be exploited for simultaneous intersexual development.

Thus I think that the behavior of the intersexes in *Bonellia* is exactly what is expected if the time law applies and if this law is tested according to the special features of the *Bonellia* case. I think that BALTZER's opposition is at least partly due to the fact that he tries to transfer the definitions made for *Lymantria* literally to *Bonellia*. In *Lymantria* one of the consequences of the time law is that organs of later differentiation will be the first to show the sexual change in intersexuality. But in *Bonellia* the whole situation is different as already explained; no seriation of the time of differentiation of the organs comes into play but only the points mentioned above.

In his endeavors to disprove the time law and to prove a simultaneous intersexual development, BALTZER needs also intermediate organs. I cannot agree that an incomplete seminal tube or proctodaeum is intermediate. The only organ which might be called intermediate in some intersexes is the coelom (narrow in males, wide in females). But an intermediate condition of an organ with only quantitative alternative is expected just as well on the basis of the time law.

There could be much said in regard to individual points in BALTZER's argumentation e.g. his allusions to the theory of genic balance

which are not very fortunate, but as I do not have any personal experience with *Bonellia* this may suffice. BALTZER has based his criticism of the time law in *Lymantria* upon what he believes to be disproof of this law in *Bonellia*. My conclusion is that he has not succeeded in proving his point even for his own object and that the intersexes in *Bonellia* are just as those in *Lymantria*, a product of the time law.

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UNTERSCHIEDE DER ZAHMEN UND WILDEN
MEERSCHWEINCHEN (CAVIA PORCELLUS LINN. UND
CAVIA RUFESCENS LUND) BEZÜGLICH IHRER
IMMUNISIERBARKEIT DURCH DIPHTERIEANATOXIN

VON

GERTA VON UBISCH und JANDYRA PLANET DO AMARAL

Instituto Butantan Brasilien

Abteilung für Veterinär u. Abteilung für Immunologie

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Die Gleichmässigkeit der Reaktion einer Tiergattung bei immunologischen und serologischen Versuchen ist eine unumgängliche Voraussetzung für ihre Verwendbarkeit. Schliessen wir doch aus dieser Reaktion in den einen Fällen auf die Stärke des Giftes, in den anderen auf den Wirkungsgrad des Gegengiftes und Heilserums. Eine *bekannte* Ungleichmässigkeit der Tiere macht sie entweder ganz unbrauchbar oder zwingt uns, den Versuch so oft zu wiederholen, dass er unökonomisch oder doch zu zeitraubend wird; eine *unbekannte* Ungleichmässigkeit kann katastrophal werden wegen der Unrichtigkeit der Schlüsse, die aus den Versuchen gezogen werden.

Selbstverständlich bedeutet „gleichmässig“ und „ungleichmässig“ immer etwas Relatives; was verlangt wird, ist, dass die Dosierung, die nötig ist, um die Ungleichheit des Tiermaterials auszuschalten, nicht so verschieden gross ist, dass die Eindeutigkeit der Resultate dadurch in Frage gestellt wird.

Die Ungleichmässigkeit ist bedingt durch die Variationsbreite des Individuums infolge ungleichmässiger äusserer Bedingungen, so Aufzucht, Futter, Klima. Einen grossen Einfluss übt das Gewicht der Tiere aus. So verwendet man für viele Diphtherieversuche stets Meerschweinchen von 300 gr, was einem Alter von etwa einem Monat

entspricht. Diese Ungleichmässigkeiten sind zum Teil ausschaltbar durch möglichst gleichmässige Tierhaltung.

Abgesehen hiervon gibt es aber auch Ungleichmässigkeiten in denen die Reaktion auf erblichen Faktoren beruht, durch die sich die verschiedenen Rassen und Varietäten unterscheiden. Beschränken wir uns hier auf die Meerschweinchenliteratur, so ist es bekannt, dass es erbliche Rassen mit unterschiedlichem Katalasegehalt gibt (5), solche, in denen sich das Fehlen resp. Vorhandensein des Complementes vererbt (4).

Wenn Rassen und Varietäten sich verschieden verhalten, so liegt der Verdacht nahe, dass verschiedene *species* noch grössere Unterschiede zeigen werden; dies wird bestätigt durch eine Arbeit von HOLZER, *Ueber die serologische Differenzierung von zwei Meerschweinchenarten*, in der die beiden Species *Cavia porcellus* und *C. rufescens*, sowie ihre Nachkommen auf ihre Bluteigenschaften hin analysiert werden. Das Resultat ist, dass mit Hilfe von Haemolysinen und Agglutininen eine Differenzierung des Blutes der beiden Species gelingt; nicht dagegen mit Praecipitinen, während Anaphylaxieversuche kein einheitliches Resultat geben. Die Mischlinge stehen zwischen den beiden Species hinsichtlich Haemolysinen und Agglutininen.

Während eine Analyse der serologischen Eigenschaften der wilden Meerschweinchen für Europa und Nordamerika hauptsächlich theoretisches Interesse beanspruchen kann, liegen die Verhältnisse in Südamerika anders, da hier die „wilden Meerschweinchen“ tatsächlich wild vorkommen. Da sie sich mit den domestizierten Meerschweinchen kreuzen lassen, durfte es in Brasilien kaum ein grösseres Bioterium geben, in denen nicht etwas „Wild“blut vorkommt. Man sieht dies leicht an der dominierenden Agoutifärbung, die bei den direkten Kreuzungsprodukten einheitlich vorkommt, aber auch in späteren Generationen bei Nichtreinagoutitieren als Scheckung auftreten kann (2). Selbst wenn man aber eine Zucht von Meerschweinchen vor sich hat, bei der jede Agoutifärbung eliminiert ist, kann man in Brasilien nie sicher sein, ob nicht andere Wildcharaktere in den Tieren vorhanden sind. Ein sehr günstiges Merkmal, in dem sich domestizierte und wilde Meerschweinchen unterscheiden, sind die Schädelnähte, die schon lange als diagnostisches Merkmal benutzt wurden und die in der Arbeit von DETLEFSEN (2) genauer analysiert

sind. Abb. 1 und 2 gibt die Schädel eines wilden und zahmen Meerschweinchens wieder, aus denen die Verschiedenheit der Suturae naso-frontales und frontoparietales ohne weitere Beschreibung hervorgeht. DETLEFSEN fand, dass die Schädelnähte von *Cavia rufescens* dominieren, in der F_2 und in den folgenden Generationen treten neben den Formen der Eltern intermediäre Formen auf.

Solche Zwischenformen findet man nun auch in äusserlich ganz reinen zahmen Meerschweinchchen des Bioteriums von Butantan, wo vor etwa 11 Jahren wilde Meerschweinchchen hereingekreuzt wurden und wo sich in einigen Ställen einfarbige Agoutizuchten finden, während in anderen alle möglichen Farben vorkommen, andere wieder

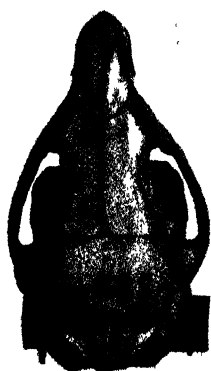


ABB 1 Schädel von *Cavia rufescens* „Preá“

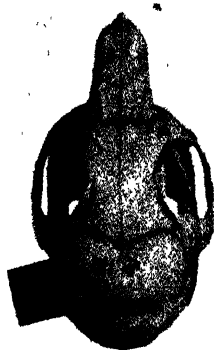


ABB 2 Schädel von *Cavia porcellus* „Meerschweinchen“

äusserlich rein domestiziert sind. Die Unabhängigkeit der Vererbung der verschiedenen Merkmale schliesst also hier ein grosses Gefahrmoment ein, falls eine serologische Verschiedenheit auftreten sollte.

Da die wilden Meerschweinchchen, *Cavia rufescens*, in Europa ziemlich unbekannt sind, möchten wir sie in einem Bilde wiedergeben Abb. 3 und kurz beschreiben, ehe wir zu unseren Versuchen übergehen.

In Südamerika gibt es eine grosse Anzahl verschiedener Species wilder Meerschweinchchen, die in Brasilien alle Preá genannt werden: *Cavia aperca*, *culleri*, *rufescens* u.s.w. Sie sind in Grösse und Farbe

etwas verschieden, doch scheinen sie alle miteinander kreuzbar zu sein (ebenso wie mit den zahmen Meerschweinchen), wodurch ihre Formenmannigfaltigkeit gross und ihre Diagnostizierbarkeit sehr erschwert wird.

Die Species, die im Staate São Paulo wohl am häufigsten ist und mit der wir gearbeitet haben, ist *Cavia rufescens* LUND, oder dieser Gattung doch sehr nahe stehend. Es sind etwa 25 cm lange Tierchen, die einem Wiesel ähnlicher sehen als einem Meerschweinchen; ihre Farbe ist schwarzbraun, d.h. die einzelnen Haare sind an der Basis grau, der grösste Teil schwarz mit einer schmalen gelben



ABB 3 *Cavia rufescens* „Préá.“

Spitze: durch diese Zonen kommt die Agoutifärbung zustande. Die Bauchseite ist einheitlich graugelb. Die Tierchen sind sehr scheu, verschwinden sofort, wenn man in ihre Nähe kommt. Wenn sie schwer zu fangen sind, so ist dies weniger eine Folge ihrer Schnelligkeit als vielmehr des Terrains, auf dem sie leben: einem gestrüppreichen Camp, wo sie sich leicht verbergen oder in ihre Gänge schlüpfen können. Sie werden von den Wald- und Campbewohnern gegessen und zu diesem Zwecke mit Hunden gejagt, Will man sie lebend fangen, so kreist man sie mit Feuer ein.

VERSUCHE

Da Meerschweinchen sich ungemein gut gegen Diphterie immunisieren lassen, haben wir uns als erste Aufgabe die gestellt, die Wirksamkeit des Diphterieanatoxins bei Meerschweinchen und Préás

zu vergleichen. (Diphtherieanatoxin ist eine Mischung von Diphtherietoxin und Formol, die trotz ihrer atoxischen Wirkung in geeigneten Versuchstieren Antikörperbildung hervorruft).

Unsere Versuche wurden bisher an 8 Meerschweinchen und 5 Präas ausgeführt, von denen 5 Meerschweinchen und 3 Präas eine, 3 Meerschweinchen und 2 Präas eine zweite Serie darstellen. Als Antigen wurde das Diphtherieanatoxin verwendet, das im Instituto Butantan hergestellt wird und von dem 5 ccm in Meerschweinchen eingespritzt nach Verlauf von einem Monat im Serum des Tieres einen antitoxischen Titer ergibt, der im Stande ist, 10 M.L.D. eines Toxins zu neutralisieren, der in einer Verdünnung von 1 : 500 wirksam ist. 1 M.L.D. ist die kleinste Dosis von Diphtherietoxin, die nach 96 Stunden ein Meerschweinchen vom Gewichte von 250 gr. (resp. eine Taube von 300 gr.) tötet. Den Tieren wurden im Intervall von je 10 Tagen (resp. 20 Tagen, wenn dazwischen Blut entnommen wurde) 0,5 ccm; 1 ccm, 1,5 ccm; 2 ccm; 2,5 ccm des Diphtherieanatoxins durch physiol. Kochsalzlösung auf 4 ccm ergänzt, subcutan eingespritzt.

Die Dosierung wurde bei der ersten Versuchsserie — Tabelle I — durch Einspritzung der Toxin-Antitoxin Standardmischung in ausgewachsene Tauben von 300 gr vorgenommen, entsprechend der im Institute üblichen Methode (6), wobei in der Tabelle die Menge reinen Serums angegeben ist, die eine Menge 1 L + neutralisiert und im Falle der Präas, bei denen Neutralisation nicht erhalten werden konnte, die grösste Dosis des Giftes, die auf Neutralisation untersucht wurde. (1 L + ist die kleinste Menge Toxin, die mit einer antitoxischen Einheit gemischt, noch ein Meerschweinchen vom 250 gr. in 96 Stunden tötet.)

Bei der II. Versuchsserie — Tabelle II — wurde die Menge Serum festgestellt, die 1 M.L.D. (siehe oben) neutralisiert, resp. beim Präa, die grösste Menge des reinen Serums angegeben, die auf Neutralisation untersucht wurde ohne Erfolg der Neutralisation.

Die Resultate sind aus den Tabellen zu entnehmen. In der ersten Serie war die Gesamtdosis des eingespritzten Anatoxins gegeben in 5 Injektionen zu 0,5 ccm; 1 ccm; 1,5 ccm; 2 ccm; 2,5 ccm gleich 7,5 ccm, die maximale Dosis 2,5 ccm. In der zweiten Serie wurden nur 3 Injektionen zu 0,5 cc; 1 ccm; 1,5 ccm in einer Gesamtmenge von 3 ccm gegeben mit einer Maximaldosis von 1,5 ccm.

Die absolute Menge des reinen Serums, das in der ersten Serie eine Dosis L + neutralisiert, ist bei den Meerschweinchen 0,5 ccm, während bei den Preás die doppelte Menge die Dosis L + nicht neutralisierte.

In der zweiten Serie war die Menge Serum, die 1 M.L.D. neutralisiert, bei den Meerschweinchen 0,1 ccm, während bei dem einen Preá — 135 — die funnfache Menge; bei dem anderen — 144 — die zehnfache Menge des Serums zur Neutralisation nicht ausreichte; (Grössere Mengen des Serums wurden nicht auf Neutralisation untersucht).

Aus diesen Versuchen ergibt sich ein deutlicher Unterschied im Verhalten der beiden Species, *Cavia porcellus* und *C. rufescens* bezüglich ihrer Immunisierbarkeit durch Diphtherieanatoxin. Die zahmen Meerschweinchen reagieren leicht auf eine antigenische Erregung, während die wilden Meerschweinchen sich resistent zeigen.

Die geringe Zahl der Versuchstiere veranlasst uns, das Resultat immerhin mit einigem Vorbehalt zu geben, da die Möglichkeit nicht auszuschliessen ist, dass sich auch Preás finden könnten, die sich besser immunisieren lassen als die hier verwendeten 5 Preás. Die Ergebnisse bei diesen Tieren jedoch durften als sichergestellt gelten, da es sich ja bei einem Tier nicht um eine einzige Blutentnahme und Prüfung handelt, sondern von der zweiten Injektion an eine Blutentnahme mit einer Injektion abwechselten. So haben wir es tatsächlich hier mit 16 Neutralisationsversuchen zu tun, die sämtlich negativ verliefen.

Eine Ergänzung zu diesen Versuchen gibt eine kleine Versuchsserie mit je zwei Meerschweinchen und zwei Preás vom gleichen Gewicht (Meerschweinchen 343 und 305 gr; Preás 345 und 305 gr) die gleichzeitig eine Einspritzung von je 1 ccm Diphtherietoxin vom Verdünnungsgrad 1 : 300 erhielten. Alle 4 Tiere starben nach 2–3 Tagen unter den Symptomen einer Diphtherieintoxication. Sollte dies Ergebnis sich als allgemein gültig erweisen, so würde es gemeinsam mit den obigen Ergebnissen besagen, dass die wilden Meerschweinchen ebenso empfindlich sind für Diphtheriegift wie die zahmen, dass ihnen aber die Antikörperbildung abgeht.

Die Versuche sollen mit grösseren Zahlen von Tieren wiederholt werden, die Art der Vererbung an ihren Mischlingen geprüft, sowie das Verhalten der Tiere anderen Krankheiten und Giften gegenüber untersucht werden.

TABELLE 1

Serie I

Absolute Menge des eingespritzten Antigens 7 ccm	
Maximale Menge des eingespritzten Antigens 2,5 ccm	
Meerschw. Nr.	
69, 99, 110, 116, 130	absolute Menge des reinen Serums, das 1 L + neutralisiert = 0,5 ccm
Preás Nr.	
95, 117, 175	1 ccm reines Serum neutralisiert 1 L + nicht.

TABELLE 2

Serie II

Absolute Menge des eingespritzten Antigens 3 ccm	
Maximale Dosis des eingespritzten Antigens 1,5 ccm	
Meerschw. Nr.	
103, 167, 139	Absolute Menge des reinen Serums, das 1 M.L.D. neutralisiert = 0,1 ccm
Preás Nr.	
144	1 ccm reines Serum neutralisiert nicht 1 M.L.D.
135	0,5 cm reines Serum neutralisiert nicht 1 M.L.D.

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MEIOSIS IN THE POLLEN MOTHER CELLS OF *CANNA GLAUCA* "PURE YELLOW"

by

F. J. M. OFFERIJNS

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Canna glauca "pure yellow" has been obtained as a F_3 -plant from crossing *Canna glauca* "Java" · *Canna glauca* "Montevideo"; this form is remarkable for the total absence of red spots in the flowers (see HONING 1933).

In my publication. Meiosis in the pollen mother cells of some *Cannas* (OFFERIJNS, 1936) *Canna glauca* "pure yellow" has been mentioned with a few words only. Although the reduction-division of the pollen mother cells of the "pure yellow" may proceed generally in a regular manner, as already described for *Canna glauca* "Java", sometimes I found irregularities; therefore I return to this subject to give a description of the meiosis.

METHOD: The ordinary smear methods are rather unsatisfactory for the study of the pollen mother cells of *Canna*; the prophase-chromosomes can rarely be seen satisfactorily in aceto-carmin owing to the heavy staining of the cytoplasm, so that an exact judgment of the stage of development is impossible, or at least very difficult. The study of the root-tips too (with the "Koch-methode" of HEITZ, 1935) rarely give results, in this case owing to the swelling of the chromosomes.

The investigation of newly collected material has given a better idea of the moment that the normal development of the pollen mother cells is disturbed by some preceding irregularity, so that the metaphase-stage cannot be reached. In metaphase and anaphase of the meiotic divisions further irregularities do not occur; if the metaphase-

stage (I) has once been reached, the division proceeds in the regular manner.

SOMATIC TISSUE: Generally the diploid number of chromosomes could easily be counted in cells of the wall of the anther ($2n = 18$). No clear morphological differences were to be found between the chromosomes in metaphase-plates, which are most suitable for countings; the investigation of other somatic tissue did not yield better results in this connection. In sections of the top of the stem of a germinating seed it may be seen that there are differences between the chromosomes in prophase, but their minuteness is an impediment for exact analysis, the same holds good for the mitotic division in root-tips.

TAPETUM: In nuclei of the tapetal cells in the prophase of mitosis morphological differences could often be seen, but they could not be laid down with accuracy in a drawing. The connection of some chromosome with the nucleolus could not be definitely stated. Although generally the divisions of the tapetal cells seems to be brought about in a normal manner, nuclei were sometimes found with more than 18 chromosomes. These chromosomes did not differ in appearance from those in normal cells.

MEIOSIS OF THE POLLEN MOTHER CELLS: The pollen mother cells in the first stage of meiosis have large nuclei with a big nucleolus; the greater part of the nucleolus is clear and the minute threads of the reticulum are hardly visible. After reaching the leptotene-stage the chromomeres are clearly visible; the very fine leptotene-threads associate in pairs and the (homologous) chromomeres are seen to be lying in corresponding places. The transition from leptotene to zygotene proceeds gradually: pairing and paired threads are found together in the same nucleus. Soon after partial or complete pairing the contraction begins in the paired threads; they become thicker (and their staining capacity is greatly increased); meanwhile the nucleus passes in the ultimale zygotene, when large loops lie throughout the nuclear cavity. The sides of the threads become more granular in the development of the pachytene-stage. In the middle of these pachytene-threads a length-wise split becomes visible, so that the double structure of the threads is apparent again. In favourable circumstances it could be stated with certainty that in some places four strings

(chromatids) constitute this pachytene-thread: a new split has developed.

The transition from pachytene to diplotene is a rather rapid process; in diplotene the chromosomes appear as bivalents, with their four chromatids, held together by chiasmata. As the nucleus is filled up for the greater part with the diplotene threads, contraction of the crossed and twisted chromatids begins; the condensation of the bivalents does not take place simultaneously, for each of their chromatid-pairs already has a very condensed portion, while the other parts are still in the diplotene-stage. All stages pass gradually from one to the other. Generally the earlier condensed part of the bivalents is not in the middle of the chromatid pairs but nearer to one end. The condensation is a spiral-wise contraction of the chromatid-pairs. Apparently, there is a movement of the chiasmata away from the condensing portion towards the slender distal part, which still clearly shows the diplotene-threads.

As may be concluded from my preparations in many pollen mother cells normal diakinesis has not been reached, for several times I found that nearly all pollen mother cells of a loculus had perished in this stage. (Such a loculus is afterwards empty, whereas another is filled up with pollen in the ripe stamen). It is clear that these pollen mother cells have no effect whatever on the development of the pollen. There is some irregularity or disturbance that obstructs the normal transition from pachytene to metaphase. If a pollen mother cell has once reached the I metaphase-stage, division further proceeds normally. It is obvious, therefore, that the ripe and fertile pollen has been developed in a normal manner, and it elucidates the fact, as mentioned by HONING (1928) that with *Canna* "sometimes in young not yet opened flowers the theca is empty, whilst that of the next flower is very well supplied" and that "it also occurs that one loculamentum has no pollen and the other of the same theca is provided with it". With *Canna glauca* "pure yellow" I found no abnormalities in the further stages of meiosis, neither in metaphase nor in anaphase (I and II). The metaphase-plate I is regular: nine bivalents are evident; anaphase is a regular process too, although the separation of the chromosomes is not always simultaneous. In early anaphase I the fine connections are to be seen between the arms of the chromosomes; they do not break simultaneously for the two arms.

On one slide an abnormal metaphase I has been found with 18 chromosomes (univalents?) in a spindle that was partly double. It is not clear what has been the origin of such a form and what may be the further development. BLEIER (1930) mentions in a critical discussion that with *Canna-hybrids* already GUYER (1902) has observed "Doppelspindeln". Undoubtedly GUYER means then two separate spindles (as appears from a drawing in his publication on pigeon-hybrids); I did not meet with such totally double spindles in metaphase I.

SUMMARY

1. The pairing of the single leptotene-threads in *Canna glauca* "pure yellow" is evident.

2. A new split (the acquation-split) is in favourable cases already visible in the pachytene-stage.

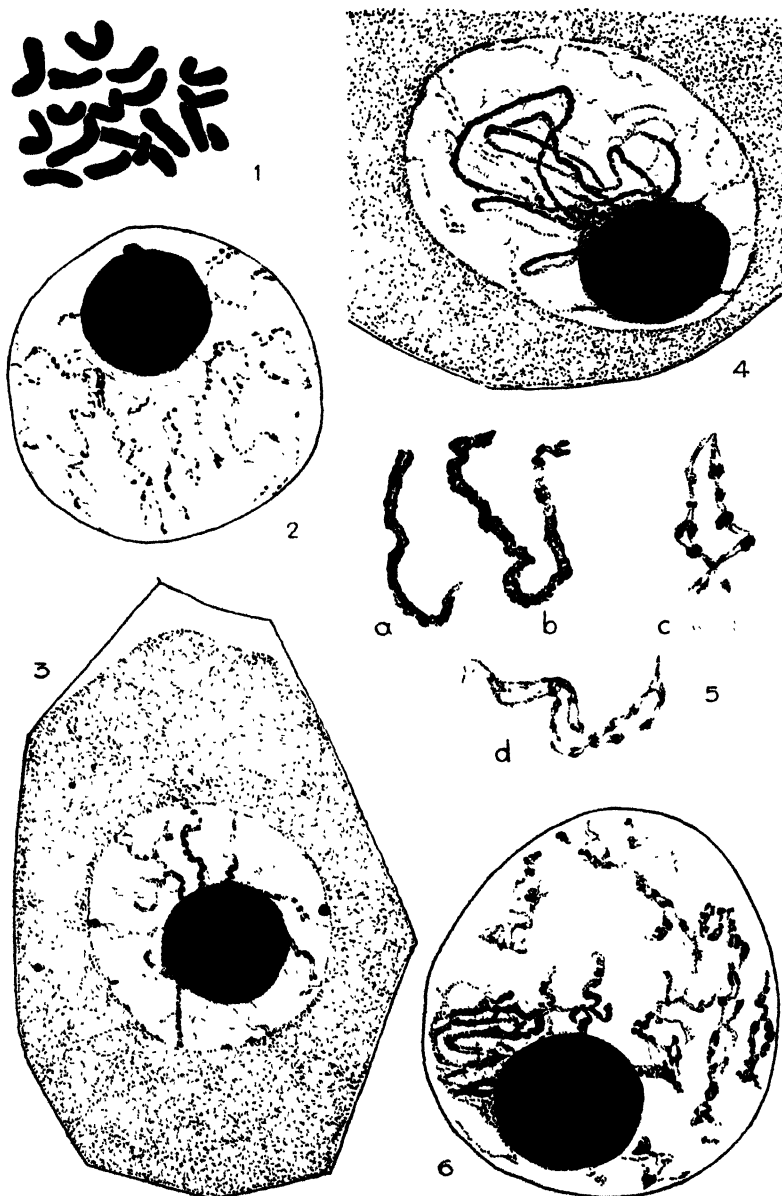
3. A greater portion of the pollen mother cells, after having reached the diplotene-stage, perishes (before diakinesis); the causes are not to be ascertained.

4. Abnormalities in metaphase I are very seldom found (partly double or forked spindles).

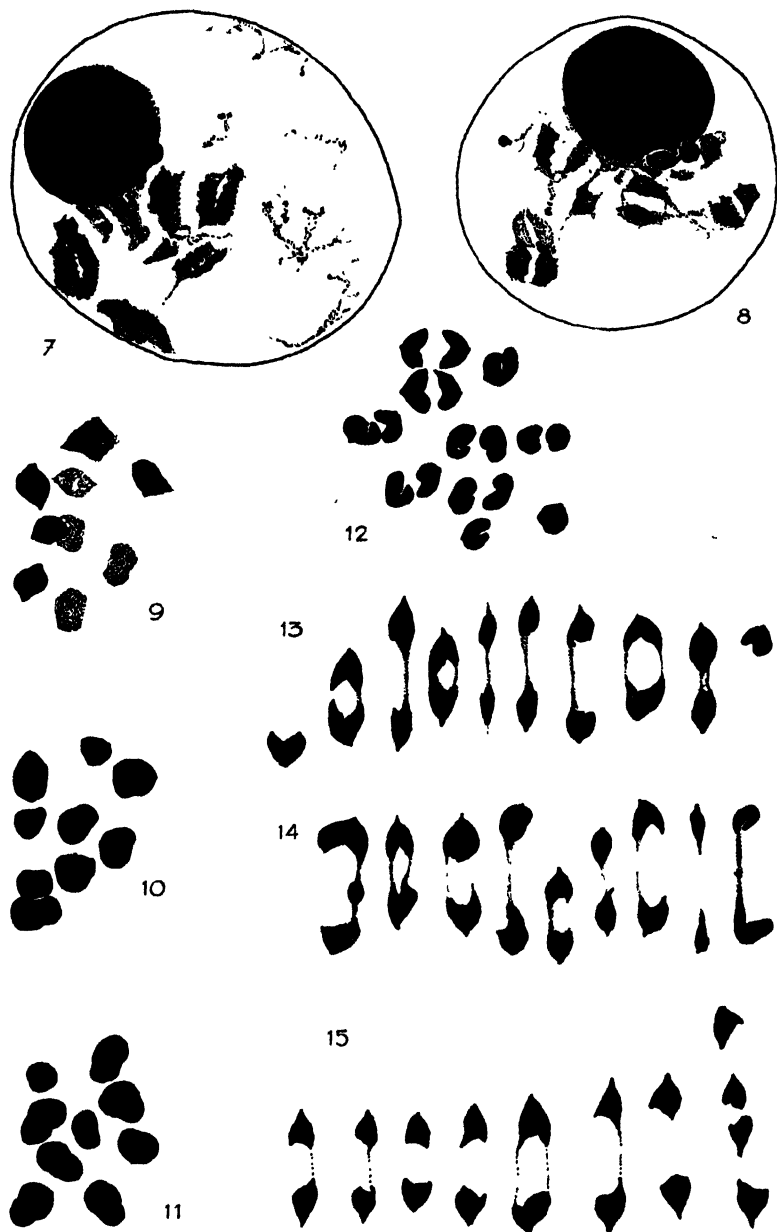
5. The pollen is formed by normal divisions; the irregularity in prophase has no influence whatever on the formation of the pollen.

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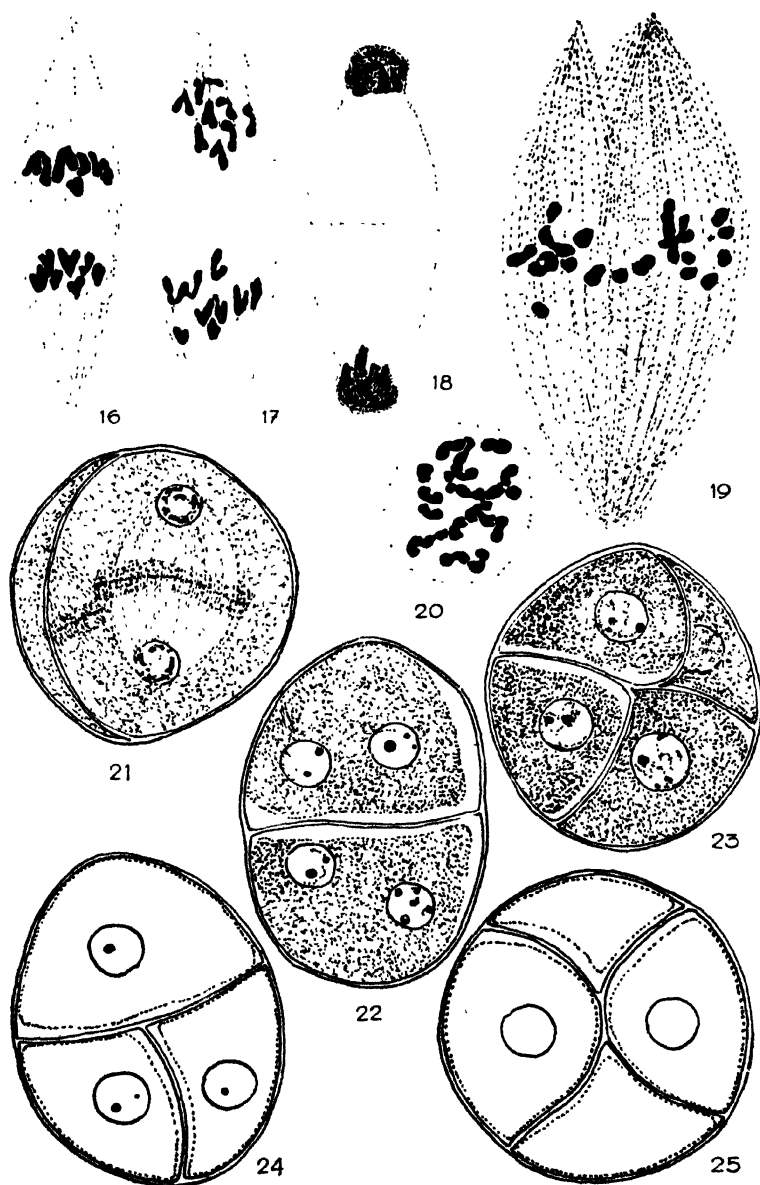
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Canna glauca "pure yellow". FIG. 1. Somatic metaphase, 3600 \times . FIG. 2. Early zygotene, 1800 \times . FIG. 3. Mid-zygotene, 1800 \times . FIG. 4. Late zygotene, 1800 \times . FIG. 5. a—b—c—d, Transition from pachytene to diplotene. FIG. 6. Early diplotene, 1800 \times .



Canna glauca "Pure Yellow". FIG. 7-8. Diplotene, 1800 \times . FIG. 9-11. Metaphase I, 3600 \times . FIG. 12. Anaphase I, 3600 \times . FIG. 13-15. Anaphase I, 2700 \times .



Canna glauca "Pure Yellow". FIG. 16-18. Ana- and telophase II, 1800 \times . FIG. 19-20. Abnormal spindle, 1800 \times . FIG. 21-25. Development of the tetrads, 600 \times .

SEVENTH INTERNATIONAL CONGRESS OF GENETICS

In accordance with a resolution of the International Committee and with the decision of the Organising Committee elected by the Genetical Society of Great Britain, the VIIth INTERNATIONAL CONGRESS OF GENETICS will meet in Edinburgh in 1939, probably from August 23rd-30th inclusive. Professor F. A. E. Crew, Institute of Animal Genetics, University of Edinburgh, Edinburgh, 9, has been appointed General Secretary to the Congress and to him all correspondence concerning it should be addressed.

Prof. F. A. E. Crew

CHROMOSOME NUMBERS AND SPERMATOGENESIS IN SOME SPECIES OF THE HYMENOPTEROUS FAMILY CYNIPIDAE *)

by

K. S. DODDS, Ph. D.

Armstrong College, Newcastle upon Tyne

Department of Botany, with Genetics

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INTRODUCTION

Since the Gall-Wasps (*Cynipidae*) show the greatest diversity in modes of reproduction of all Hymenoptera, it would seem that studies of the cytological events of their life-histories should greatly assist the understanding of natural parthenogenesis.

Only few cytological data are available. Originally, HENKING (1892) and SCHLEIP (1909) investigated the cytology of the egg in the thelytokous species *Rhodites rosae*. Later, DONCASTER (1910-11-16) studied the gametogenesis of *Neuroterus baccarum*, and more recently HOGGEN (1919-20) traced the differentiation and nuclear history of the oocytes of *Neuroterus numismalis* agamic form *numismalis*, *Rhodites rosae* and *Cynips Kollari*.

The observations described below were made as part of a program-

*) [Some years ago, the late Professor L. DONCASTER F R S. asked me to continue his work on the *Cynipidae*. Owing to the pressure of work in other directions, I was obliged to decline. However, I promised that, as soon as I had a student capable of carrying out the research, the work would be resumed. In Dr. K. S. DODDS, I found such a student, and the accompanying paper represents the earliest fruits of his labours. It is dedicated to the memory of Professor DONCASTER. — J. W. HESLOP HARRISON].

me of research of which the chief aim is to extend the work of DONCASTER and HOGBEN.

At the outset, the writer wishes to express his indebtedness to Professor J. W. HESLOP HARRISON, D.Sc., F.R.S., under whose direction this investigation has been conducted, and to Dr. K. B. BLACKBURN, D.Sc., for aid in the collection of material.

MATERIAL AND METHODS

Galls were collected in the field and stored in dry Sphagnum moss in a cool greenhouse until such time as the tenants were at a stage suitable for dissection and fixation.

Preparations from young larvae gave chromosome counts in various tissues, and gonads of unpigmented male pupae gave all stages in spermatogenesis from spermatogonia to nearly ripe sperms.

Larvae were fixed in BOUIN's picro-formol without urea (sat. aq. picric acid 15, 40% formalin 3, glacial acetic acid 2). Pupae were fixed in either BOUIN's picro-formol without urea or PEIERUSKEWITSCH's modification of GILSON's fluid. The latter was more satisfactory when details of the spindle-figure were required. Material was cleared in xylol and embedded in paraffin wax, M.Pt. 56-57°C. All sectioning was done at 10 μ .

HEIDENHAIN's iron haematoxylin stain was used in the majority of preparations, but gentian violet was also used with success. SMITH's (1934) method of using picric acid with the stain was employed; sections required two hours in the stain.

FEULGEN's (1924) "Nuclearreaktion" method for the absolute determination of chromatin was used to determine the nature of the plasmosomes in the male germ-cells.

CYTOLOGICAL OBSERVATIONS

Chromosome numbers in the Cynipidae

All the species investigated have the same haploid number of chromosomes, $n = 10$.

1. *Neurolecrus baccarum* LINNAEUS.

(Material from Raincliff Woods, Scarborough, Yorkshire).

a. agamic form lenticularis OLIVIER.

Fig. 1 illustrates the complement of chromosomes in the agamic female. The diploid number (20) is found throughout hypodermal cells, nerve cells, connective tissue, oogonial and follicle cells. The chromosomes have no characteristic morphological features and, owing to the gradation in size from the shortest to the longest member of the complement, it is difficult to carry out comparative work based on size variations. Mitosis appears to take place with perfect regularity for no lagging chromosomes are observed in side-views of anaphase stages. A tendency to somatic pairing is shown (Fig 1).

b. bisexual form baccarum LINNAEUS

Good metaphase plates are rare, so that in most cells the exact number of chromosomes cannot be determined, although it is possible to judge whether the haploid or diploid number of chromosomes is present. The following general conclusions have been reached:

In the female, hypodermal, nerve, fat and connective tissue cells all have the diploid number (20) of chromosomes. The follicle and terminal filament cells of the ovary also have 20 chromosomes, but the cells of the ovary sheath are tetraploid, as are cells of the tracheal walls.

In the male, hypodermal and fat cells have the diploid number of chromosomes. Nerve cells and connective tissue cells remain haploid. Tracheal wall cells, and cells of the testis sheath, have not been observed dividing.

Occasional tetraploid cells are observed in the hypodermal cells of both male and female. In both sexes it was found impossible to estimate even approximately the number of chromosomes in giant cells such as oenocytes.

From the foregoing it will be seen that, although the haploid-diploid ratio is maintained in nerve cells and connective tissue of male and female larvae, no general conclusion as to the maintenance of such a ratio throughout the soma is warranted. SANDERSON (1932), working with a member of the Tenthredinidae, concludes that the haploid-diploid ratio is maintained in the male and female adults of that species. The fortuitous occurrence of polyploid cells in diploid tissue leads the present writer to the opinion that no such balance is to be expected.

The chromosome complements in a haploid cell of the male and

in a diploid cell of the female are shown in Figs. 2 and 3 respectively. There is a very definite suggestion that the haploid complement is made up of five pairs; if such were the case, four chromosomes of each type would be expected in the complement of a diploid cell of a female. Unfortunately, no safe conclusion can be reached on this point; it appears that there are at least four chromosomes of any one length in the diploid complement, but the very gradual gradation in size from the shortest to the longest member, and lack of characteristic morphological features in the chromosomes, make it difficult to carry the analysis further. A careful analysis of the somatic chromosomes is being conducted at present, with the hope of solving this problem.

2. *Neuroterus numismalis* OLIVIER.

(Material from Tenby, Pembrokeshire, South Wales).

A metaphase plate from a hypodermal cell of an agamic female is shown in Fig. 4: 19 chromosomes may be counted. No plate has been found showing a greater number of chromosomes than this. If more prepared material were available however, it seems highly probable that the chromosome number would be determined conclusively as 20. This assumption seems justified from a consideration of the facts that the base number for other species of the Cynipidae has proved to be 10, and that earlier workers (DONCASTER 1910-11-16 and HOGBEN 1919-20) found it necessary to deduce the somatic numbers in the species upon which they worked from an analysis of the plates counted. The chromosomes of *Neuroterus numismalis* resemble closely those of *Neuroterus baccarum*. Distinguishing morphological characteristics are absent, and the same size-range and gradation from the smallest member of the complement to the largest are shown.

3. *Biorrhiza pallida* OLIVIER.

(Galls collected at Wylam-on-Tyne, Northumberland).

A small cluster of the root galls were collected, and of these only two were opened to use the larvae for somatic chromosome preparations. The sectioned larvae gave only one metaphase plate in which the chromosome number could be estimated approximately. The plate, cut by the microtome knife so that chromosomes are on two sections, is drawn in Fig. 5. As far as can be estimated, 20 chromosomes are present; this estimate is confirmed by the counts obtained in the spermatogenesis of the bisexual form and reported upon below.

4. *Andricus collaris* HASTIG.

(Galls collected at Birtley, County Durham).

A metaphase plate in an oogonial cell of the bisexual female is illustrated in Fig. 6. 20 chromosomes are present. As with other species described, the chromosomes show no distinguishing morphological features and little size variation.

5. *Andricus fecundatrix* MAYR.

(Material from Raincliff Woods, Scarborough, Yorkshire).

A few galls of the agamic generation were collected, but no attempt to rear the adults was made as a period of three years elapses before imagines emerge. The larvae were removed and fixed. Fig. 7 shows the only metaphase plate obtained in which the number of chromosomes can be counted with certainty — 20 are present.

6. *Aulacidea hieracii* BOUCHÉ.

(Galls collected at Birtley, County Durham).

Fig. 8. shows the chromosome complement in a hypodermal cell of a larva which was too young for its sex to be determined. The chromosomes, 20 in number, are small and show little size variation. It is worthy of note that haploid cells have not been observed in any of the preparations of somatic tissue.

Spermatogenesis

Spermatogenesis has been studied in the following species, in all of which the haploid chromosome number is 10:

I. Primitive bisexual species.

Aulacidea hieracii.

Xestophanes potentillae RETZ.

II. Bisexual generations of species showing an alternation of generations.

Neuroterus baccarum bisexual form *baccarum*.

Biorrhiza pallida bisexual form *pallida*.

Andricus collaris bisexual form *curvator*.

Trigonaspis megaloptera bisexual form *megaloptera* PANZER.

A strong similarity in behaviour between all the species has been found, so that needless repetition may be avoided by describing the spermatogenesis of *Neuroterus baccarum* in detail, and confining mention of the remaining species to points of deviation from the behaviour recorded for that species.

Neuroterus baccarum bisexual form *baccarum*.

Development of the larvae in the galls is very rapid; in about a fortnight young larvae pass to white pupae. The follicles of the testis in the full grown larvae and very young pupae contain primary spermatocytes, but when pigmentation has begun in the body of the pupa, the meiotic phase is over, and the testis contains only spermatids and nearly ripe spermatozoa. Thus it is in pupa showing no pigmentation other than in the eyes that the various maturation stages are found.

The account of the meiotic process which follows agrees very closely with that given by DONCASTER (1910) for this species.

The primary spermatocytes are rounded cells lying free in the cavity of the follicle. A large nucleus occupies most of the volume of the cell, which frequently shows one or two deeply stained granules in its cytoplasm. The resting nucleus has a slightly granular appearance and the nucleolus cannot be seen (Fig. 9). At the onset of maturation, the nucleus shows a faintly stained granular reticulum which later is transformed into thin interwoven threads. Condensation proceeds until the threads are much thicker and finally appear as chromosomes, ten in number. These are long bands, clearly double, and often with a more or less regular meridional arrangement (Fig. 10). DONCASTER (1910) observed this condition in the 'prophase' of the second division, but careful study has convinced the writer that such an arrangement is more typical of the prophase of the first division. Meanwhile the cell has become pear-shaped and the nucleus with the chromosomes fully contracted passes to the broader end. Centrosomes make their appearance in the cytoplasm and are seen, one at the apex, and the other close to the nuclear membrane at the broad end of the cell. The nucleus now elongates and becomes attenuated in the direction of the apical centrosome; the membrane remains intact. During these changes the chromosomes are loosely aggregated at the equator of the nucleus, but, when the pear-shaped form is assumed, the chromosomes contract and form a clump at the broad end of the nucleus (Figs. 11 & 12). Later, the nucleus returns to its resting condition by reassuming an oval shape, and by a gradual loosening and dispersal of the chromatin in the compact mass (Fig. 13).

While the nucleus is returning to the resting stage which is inter-

calated between the abortive and second maturation divisions, DONCASTER (1910) reported that a small cytoplasmic bud containing a centrosome is nipped off from the apex of the cell. WIEMAN (1915) likewise records the extrusion of a "polar body" at this stage in *Dryophanta erinacei* MAYR. The writer has failed to observe such behaviour in any of the species investigated; fragments of cytoplasm scattered among the spermatocytes simulate the appearance of cytoplasmic buds. Moreover, SANDERSON (1932) studied the spermatogenesis of the saw-fly *Pteronidea (Nematus) ribesii* Scop., and failed to find the extrusion of cytoplasmic buds. She suggested that the fragments of cytoplasm might be sections through the cytoplasmic bridges by which the spermatocytes are bound together.

Andricus collaris bisexual form *curvator* is the only one of the species examined in which an occasional intranuclear division of the chromatin during the first division has been observed. Fig. 14 shows a metaphase plate formed at such a division, and Fig. 15 illustrates an attempted anaphase in which no spindle-mechanism is developed. Both preparations are from the same follicle. DONCASTER (1910) occasionally observed the division of the chromatin inside the nucleus in *Neuroterus baccarum*, and suggested that it might possibly be the persistent remnant of a true nuclear division, or comparable to the "intranuclear karyokinesis" described by KOSTANECKI (1904) in parthenogenetic eggs of *Mactra*.

It is difficult to determine the precise nature of the spindle-mechanism developed during the first division, although all workers on the Hymenoptera, have reported fibrillar connections between the apical centrosome and the nuclear membrane. DONCASTER (1910), pp. 92-93, writes, "in some cells the narrow apex of the cell is elongated into a fine process, with the centrosome (centriole) at its tip, like that figured by MARK and COPELAND in the corresponding stage in the Bee. Fine threads run down from this to the nucleus, but it is difficult to determine whether they penetrate inside the membrane or pass outside it, for, at the narrow end, the nuclear membrane becomes indistinct and confused with these fibres, while remaining clearly defined at the opposite wider pole". The present investigator has studied this stage carefully and concluded that the nuclear membrane remains intact. A cone of indistinct spindle-fibres stretches from the apical centrosome to the membrane through which

the fibres do not penetrate. At the wider pole the nucleus approaches so near to the edge of the cell, that one cannot determine whether or not the spindle-fibres entirely surround the nucleus and stretch from pole to pole.

After the resting stage which supervenes between the abortive first and equational second division, the chromatin reappears as a granular reticulum very similar to that of the primary spermatocyte. Condensation proceeds, and the reticulum gives place to an indeterminate number of rods and granules from which ten very definite double bands of chromatin arise (Fig. 16). The chromosomes, each showing a longitudinal, median split are arranged later on the equatorial plate, and at about this time the nuclear membrane disappears. In the metaphase plates of *Neuroterus haccarum*, *Biorrhiza pallida*, *Trigonaspis megaloptera* and *Andricus collaris*, the chromosomes show well-marked secondary pairing (Figs. 17, 18, 19 and 14). This condition can be seen in DONCASTER's Fig. 12 of the metaphase at the second division in *Neuroterus haccarum*.

Material fixed in BOVIN's picro-formol without urea does not lend itself to a study of the spindle-figure. Spindle-fibres radiating from the poles to the chromosomes are visible, but no centrosomes can be seen. With PETRUNKEWITSCH's modification of GILSON's fluid however, the spindle-figure is very prominent and the two centrosomes are seen, one at each pole. Anaphase is quite regular, and the spindle-figure extends the whole length of the cell. The centrosomes at the extremities of the cell are still visible during telophase and lie very close to the condensed daughter nuclei, between which a sheaf of spindle-fibres extends until the separation of the two daughter cells (Fig. 21). The heavily stained, structureless clump of chromatin of the daughter nucleus opens up and is dispersed around the periphery of the nuclear membrane (Fig. 22). Thus the primary spermatocyte gives rise to two spermatids, each of which undergoes the cytoplasmic changes of spermateosis, and becomes a functional sperm.

Throughout spermatogenesis, from the onset of maturation to the formation of the spermatid, a small stained plasmosome is visible in the cytoplasm of the cell. The granule is situated outside the sphere of influence of the spindle-figure, and seems to be included in one of the daughter cells as a result of the division of the cytoplasm rather than by any regulated movement to one of the poles. It is not observed

with the same frequency in the different species examined as will be seen from the following table:

<i>Neuroterus baccarum</i>	throughout all stages.
<i>Andricus collaris</i>	" " "
<i>Trigonaspis megaloptera</i>	" " "
<i>Biorrhiza pallida</i>	not constantly observed until after the abortive di- vision.
<i>Xestophanes potentillae</i>	observed only in a low per- centage of cells.
<i>Dryophanta erinacei</i>	not present (WIEMAN 1915).

DONCASTER (1910) tentatively suggested a connection between the granule and the inheritance of sex, but his breeding experiments of 1916 showed conclusively that a scheme based on the occurrence of two types of sperms in any one male is insufficient to explain the complex cycle in *Neuroterus baccarum*. The body gives a negative reaction when tested by FEULGEN's "Nuclearreaktion" method for the absolute differentiation of chromatin. It is probably of the same type as the plasmosome seen in the growth period and spermatocyte-division of *Pentatoma* (WILSON 1913), and its nature must remain problematical.

GENERAL CONSIDERATIONS

All the species reported upon above show the hymenopteran abortive first division in spermatogenesis. The occurrence of this division in *Aulacidea hieracii* and *Xestophanes potentillae* supports the supposition that, in the tribe Aulacini, reproduction is by facultative parthenogenesis in the manner typical of most Hymenoptera. Knowledge of reproductive behaviour in the Aulacini is based entirely on biological evidence. ADLER (1881) secured experimentally the successive generations of *Aulacidea hieracii* and reported that there was no alternation of generations within that species; KINSEY (1920) has since confirmed this for several North American species of Aulacidea. BEYERINCK (1883) found that females of *Aulacidea hieracii*, which he thought were non-impregnated, could lay eggs on *Hieracium rigidum*. Galls were formed but he did not carry investigations further. If reproduction is by facultative parthenogenesis, all

eggs undergo maturation with reduction; the fertilised egg produces a female (diploid) and the unfertilised egg a male (haploid). In the larval material examined, no haploid cells were observed in other than gonial tissue.

The condition of the soma in haploid hymenopteran males has been a controversial subject since NACHTSHEIM (1913) reinvestigated the cytology of the honey-bee after the pioneer work of PETRUNKEWITSCH (1901). SANDERSON (1932) has given an able discussion of haploidy in the Hymenoptera, but the present writer cannot agree with her conclusion that the question raised by HERTWIG (1920) "as to whether the haploid number is retained in the soma" may be regarded as settled. Conditions in the soma of *Neuroterus baccarum* have been examined thoroughly, and it has been seen that only a comparatively small amount of tissue, namely connective and nerve cells, remains haploid; between male and female, a haploid-diploid or diploid-tetraploid ratio is not maintained consistently. Thus, in the Cynipidae at least, the hypothesis of TAUSON (1924) that, although the germ-plasm may be haploid, the soma may become diploid by "un processus de régulation qui rétablit le nombre diploïde de chromosomes", is far from being disproved. A study of oogenesis in *Neuroterus baccarum* is being carried out at present and it is hoped to publish the results shortly. Possibly more light will be shed upon this problem by a knowledge of behaviour during maturation of the egg which gives the male.

Somatic and secondary pairing are observed in the male larvae. Both types of pairing have been reported by many workers in plants and animals (see WILSON 1928 and DARLINGTON 1932) and are, without doubt, the indication of some degree of genetical homology between the paired chromosomes. Such relationship could be brought about by reduplication of chromosomes within the diploid set, or by polyploidy. Unfortunately, it has not been possible to arrive at a definite conclusion as to the constitution of the female diploid complement, but there are indications that it may be made up of five groups of four chromosomes. If this is so, then there is a distinct possibility that the Cynipidae are on a diploid-tetraploid basis of chromosome constitution. PEACOCK and SANDERSON (1930) find that, in the haploid male of the saw-fly *Pteronidea (Nematus) ribesii*, the chromosomes arrange themselves into three pairs and two sets of

four and four odd members. They suggest that polyploidy may have some underlying significance in this arrangement. In spite of the strong genetical evidence summarized by SPEICHER (1936) against the possibility of a solution of the reproductive problems of Hymenoptera being found in a diploid-tetraploid basis of chromosome constitution, it does seem that this aspect of the problem cannot be neglected in further studies.

SUMMARY

1. The following species have a haploid number of chromosomes, $n = 10$:— *Neuroterus baccarum*, *Neuroterus numismalis*, *Andricus collaris*, *Andricus fecundatrix*, *Biorrhiza pallida*, *Trigonaspis megaptera*, *Aulacidia lucracu* and *Nestophanes potentillae*.

2. The chromosomes in the haploid cells of the male of *Neuroterus baccarum* show somatic pairing. The chromosome complement of the female may be made up of five groups of four homologous chromosomes.

3. Spermatogenesis follows the usual hymenopteran plan. No cytoplasmic bud appears to be liberated during the first maturation division. A chromatoid body may be traced to the spermatids only in a limited number of species. It gives a negative reaction when tested by FEULGEN's "Nuclealreaktion".

4. Secondary pairing is shown at the second metaphase in spermatogenesis.

5. It is suggested that the chromosome constitution of the Cynipidae may be on a diploid-tetraploid basis.

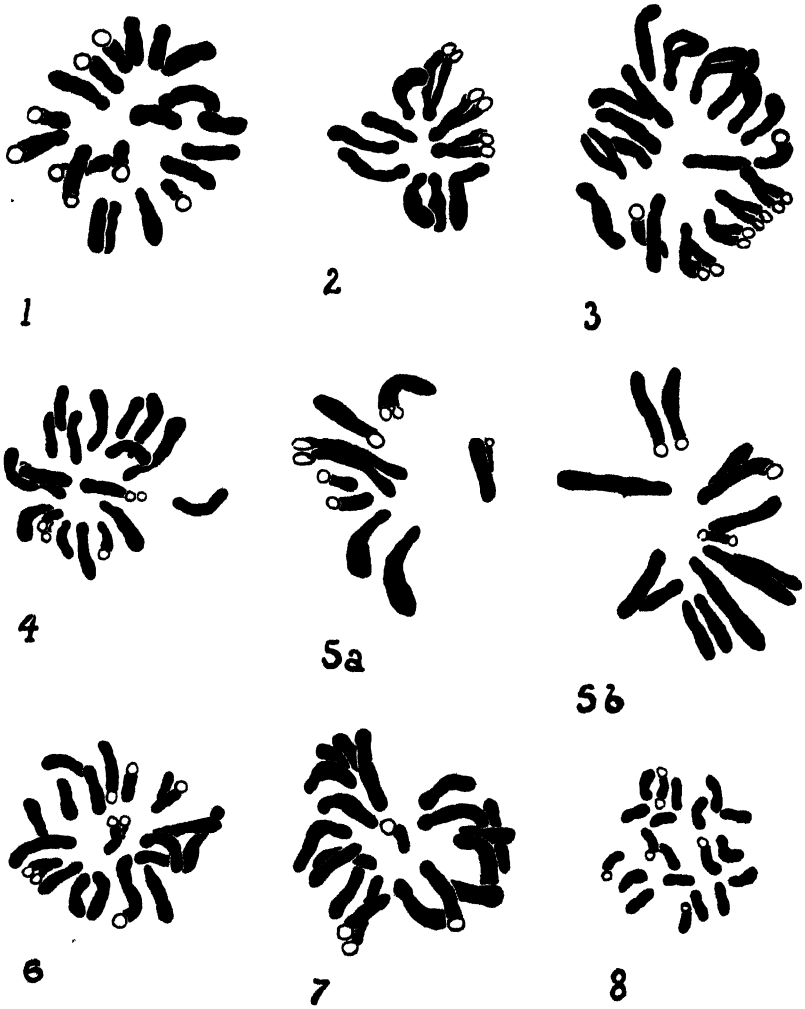
DESCRIPTION OF FIGURES

The drawings were made at a magnification of approximately 4,000, by means of a Watson Camera lucida, a Watson "Service" microscope, a Watson "Utility" $\frac{1}{12}$ oil immersion objective and an 18 compensating ocular eyepiece.

PLATE I

Metaphase plates of somatic chromosomes.

- FIG. 1. *Neuroterus baccharum* agamic form *lenticularis*. Oogonial cell: 20 chromosomes. Note tendency to somatic pairing. Bouin with urea/Haem.
- FIG. 2. *Neuroterus baccharum* bisexual form *baccharum*. Hypodermal cell: 10 chromosomes. Well-marked somatic pairing. Bouin/Haem.
- FIG. 3. *Neuroterus baccharum* bisexual form *baccharum*. Oogonial cell of female: 20 chromosomes. Note tendency to somatic pairing. Bouin/Haem.
- FIG. 4. *Neuroterus numismalis* agamic form *numismalis*. 19 chromosomes. Bouin/Haem
- FIG. 5 a) and b) *Biorrhiza pallida* agamic form *aptera*. Plate on two sections. Approximately 20 chromosomes. Bouin/Haem.
- FIG. 6. *Andricus collaris* bisexual form *curvator*. Follicular cell: 20 chromosomes. Bouin/Gentian Violet.
- FIG. 7. *Andricus fecundatrix* agamic form *fecundatrix*. Nerve-cell: 20 chromosomes. Bouin/Haem.
- FIG. 8. *Aulacidea hieracii*. Hypodermal cell: 20 chromosomes. Petrunk./Haem.



10 μ

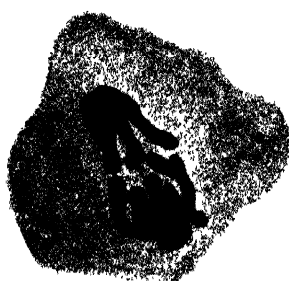
PLATE II

Spermatogenesis.

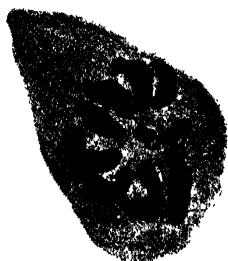
- FIG. 9. *Neuroterus baccharum*. Resting primary spermatocyte Bouin/Haem
- FIG. 10. *Neuroterus baccharum*. Spermatocyte I, showing the meridionally arranged chromosomes. The nucleus is incomplete. Petrunik/Haem
- FIG. 11. *Trigonaspis megaptera*. Abortive spindle: half-spindle of fibres radiating from apical centrosome to nuclear membrane. Bouin/Gentian Violet.
- FIG. 12. *Neuroterus baccharum*. Later stage than in Fig. 11. The chromosomes have clumped. Petrunik/Haem
- FIG. 13. *Neuroterus baccharum*. Spermatocyte II in resting stage. Bouin/Haem.
- FIG. 14. *Andricus collaris*. Spermatocyte I metaphase plate. Note the secondary pairing. This is a stage rarely found. Bouin/Haem
- FIG. 15. *Andricus collaris*. Spermatocyte I. Attempted anaphase, illustrating "intranuclear karyokinesis". Bouin/Haem



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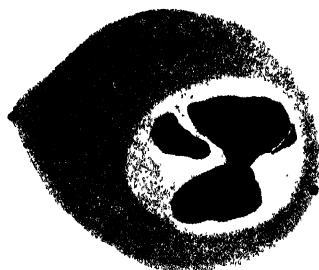
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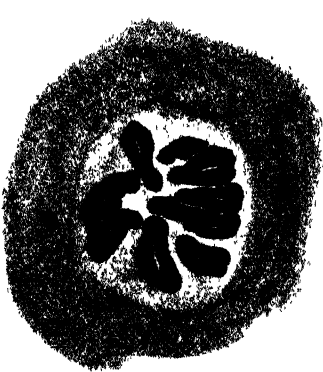
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PLATE III

- FIG 16 *Neuroterus baccarum* Spermatocyte II Emergence of doubled chromosomes prior to formation of second division metaphase plate Nucleus incomplete. Carnoy/Haem
- FIGS 17, 18, 19 & 20 *Neuroterus baccarum*, *Biorhiza pallida*, *Trigonaspis megaptera* and *Xestophanes potentillae*, respectively Spermatocytes II metaphase plates Secondary pairing well-marked in FIGS 17, 18 & 19 FIG 17: Petrunk /Haem FIG 18: Petrunk /Haem FIG 19: Bouin/ Gentian Violet FIG 20: Bouin, Haem
- FIG. 21. *Neuroterus baccarum* Telophase of second division A sheath of spindle-fibres stretches between the daughter cells, one of which receives a chromatoid body. Petrunk./Haem
- FIG 22 *Neuroterus baccarum* Spermatid containing the chromatoid body. Bouin/Haem



16



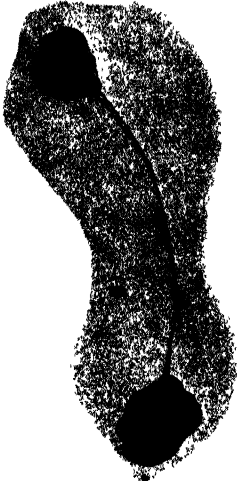
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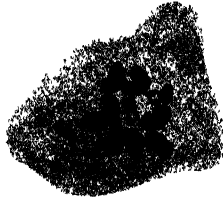
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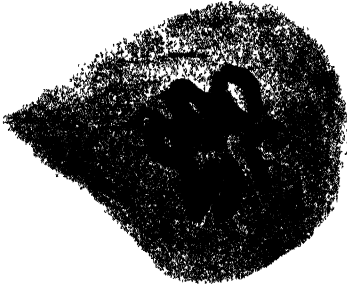
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DIE SESQUIDIPLOIDEN *NICOTIANA RUSTICA* L. \times *N. GLAUCA* GR.

von

M. F. TERNOVSKY

(Manuskript eingegangen am 6. Mai 1937)

Obgleich *Nicotiana rustica* L. und *N. glauca* GR. ein und derselben Sektion *Rustica* G. DON. — angehören unterscheiden sie sich dennoch durch eine Reihe ziemlich scharf ausgesprochener morphologischer und biologischer Merkmale. Ich führe in Kürze ihre Charakteristik an. *N. rustica* erscheint als eine verhältnismässig nicht grosse, einjährige (nach COMES manchmal zwei- und dreijährig) stark behaarte Pflanze mit gestielten Blättern und einer ziemlich breiten 2 cm. langen Kronenröhre; die Krone ist gelblich-grün, die Kapsel nicht aufspringend, mit Samen, die für diese Gattung gross erscheinen; die somatischen Zellen enthalten 48 Chromosomen.

N. glauca erscheint als eine mächtige (2–3 m. hohe) vieljährige unbehaarte Pflanze mit blaugrünen Stielblättern, einer gelben Blüte und einer schmalen 4 cm. langen Kronenröhre; die Kapsel aufspringend, mit kleinen Samen; die Zahl der Chromosomen in den somatischen Zellen = 24.

Trotzdem diese Arten systematisch einander nahstehen blieb bisher ihre Kreuzung erfolglos. KOSTOFF (1930) zählt diese Kombination zu der Gruppe, bei welcher die Befruchtung stattfindet, jedoch der Embryo sich nur im Laufe von 6–10 Tagen nach der Befruchtung entwickelt; seine weitere Entwicklung wird gehemmt und er geht zu Grunde. MC CRAY (1932) führte während seiner Studien über die Vereinbarkeit verschiedener Arten die Kreuzung von *N. rustica* var. *humilis* \times *N. glauca* aus. Die Kreuzungen ergaben entweder nicht-keimfähige Samen oder Mutterpflanzen. Derselbe Autor

stellte (1933) bei seinen Untersuchungen bezüglich der Entwicklung des Embryos fest, dass bei gegebener Kreuzung nach der Befruchtung 1—2 Teilungen stattfinden, wonach der Embryo zu Grunde geht. Diese Autoren lieferten auf embryologischer Grundlage einen Beweis dafür, dass das Erhalten von Bastarden *N. rustica* × *N. glauca* nicht möglich sei. Tatsächlich war es bis zum Jahre 1931 niemand gelungen solche Bastardpflanzen zu züchten. Die Resultate der von uns ausgeführten Arbeiten sind in Tab. 1 dargestellt.

TAB. 1. RESULTATE DER KREUZUNG *N. RUSTICA* × *N. GLAUCA*

Jahr der Bastardierung	Bezeichnung der Mutterform	Anzahl		
		der bestäubten Blüten	der erhaltenen Kapseln	der Pflanzen im folgenden Jahr
1930	var. <i>texana</i>	8	8	—
1930	„ <i>humilis</i>	9	9	—
1930	„ <i>viscosa</i>	11	11	—
1930	Erba santa	5	5	2
1931	var. <i>humilis</i>	26	25	—
1931	„ <i>viscosa</i>	13	12	2
1932	„ <i>texana</i>	2	2	—
1932	„ <i>humilis</i>	24	15	—
1932	„ <i>viscosa</i>	6	—	—
1932	Erba santa	74	41	72
1932	White barley type .	21	21	1
1933	var. <i>humilis</i>	30	28	1
1933	„ <i>viscosa</i>	10	10	4
1933	Bakunschwarz . .	34	17	3
1934		—	—	—
1934	Bakunschwarz . .	18	12	3
1934	Chmelevka	23	16	—
		314	231	88

Die in Tab. 1 angeführten Daten zeugen vor allem von der Kreuzungsfähigkeit der Arten *N. rustica* und *N. glauca*. Über die von mir im Jahre 1932 erhaltenen Pflanzen dieser Kombination berichtete ich in

Detskoje Sselo in der Abteilung für Genetik und Pflanzenzüchtung am Institut für Pflanzenbau (im Januar 1933) und in Kijew (Konferenz über Genetik und Pflanzenzüchtung im März 1933). Dr. KOSTOFF, der bei meinen Mitteilungen zugegen war, bestätigte meinen Hinweis darauf, dass bis zur letzten Zeit von *N. rustica* \times *N. glauca* keine Bastardpflanzen erhalten worden waren. Den Misserfolg



PHOT 1 Blätter: a *N. rustica* var. *viscosa*, b. F_1 *N. rustica* var. *viscosa* \times *N. glauca* (sesquidiploid), c. *N. glauca*

der vorhergegangenen Versuche erklärte er dadurch, dass er als Mutterform var. *humilis* angewandt hatte, welche auch in unsern Versuchen am schwierigsten der Bastardierung unterlag: während 4 Versuchsjahren gelang es uns nur 1 Bastardpflanze zu erhalten. Alle diese Tatsachen sprechen dafür, dass die verschiedenen Formen von *N. rustica* über eine ungleiche Kreuzungsfähigkeit mit *N. glauca* verfügen. Dabei muss aber überhaupt auf eine gewisse Schwierigkeit

hingewiesen werden, mit der das Erhalten von Bastardpflanzen verbunden ist; daher muss die Zahl der Kreuzungen genügend gross sein.

Manche Kombinationen gegebener Kreuzung offenbarten eine erstaunliche Heterosis; so erreichten im Jahre 1933 einzelne F_1 Pflanz-



PHOT 2 Blüten: *a* *N. rustica* var *viscosa*, *b* F_1 *N. rustica* var *viscosa* \times *N. glauca* (Sesquidiploid), *c* *N. glauca*

zen von Erba santa \times *glauca* 3.8 m. Höhe. Die Mutterform dieser Kombination war 60 cm., und *glauca* gegen 2.5 m. hoch.

In Übereinstimmung mit der Aufgabe vorliegender Arbeit beabsichtige ich hier meine Besprechungen auf die Polyploiden zu beschränken (Phot. No. 1 u. 2). Bis jetzt wurden in der

ersten Generation 4 solche Polyploiden erhalten. Sie haben alle 1 Genom *rustica* und je 2 Genome *glauca* und erschienen also als Sesquidiploiden¹⁾ der Formel $24r \times 12g$. Zwei von ihnen wurden im Jahre 1932 nach Kreuzung mit var. *viscosa* festgestellt und die zwei andern im Jahre 1935 nach Kreuzung mit der Zuchtsorte Bakun schwarz. Es muss vorausgesetzt werden, dass die Zahl der Polyploiden bedeutend grösser sein würde, wenn es gelänge eine grosse Anzahl von Pflanzen der ersten Generation zytologisch zu untersuchen. Von den 6 Pflanzen welche untersucht wurden, stellten 2 gewöhnliche digenome Bastarde mit je 36 Chromosomen in den somatischen Zellen vor (Abb. 1); die übrigen 4 erwiesen sich als Sesquidiploiden mit je 48 Chromosomen in den somatischen Zellen (Abb. 2), von denen augenscheinlich 24 Chromosomen *N. rustica* und 24 Chromosomen (verdoppeltes Genom) *N. glauca* angehören.

Die Reduktionsteilung geht bei den Sesquidiploiden bedeutend regelmässiger von statten als bei einem gewöhnlichen Bastard. Während beim letzteren die Chromosomen in der Metaphase der heterotypischen Teilung keine äquatoriale Platte bilden sondern in dem Plasma zerstreut auftreten, befinden sich bei den Sesquidiploiden die meisten Chromosomen in der Äquatorialebene (Abb. 3–6). Die allgemeine Konfiguration der Metaphase der ersten Teilung, die Anordnung der Chromosomen während derselben, weisen auf eine grössere Regelmässigkeit der Teilung hin. Die Anzahl der nicht in die allgemeine Fläche eingeschlossenen Chromosomen schwankt zwischen 4–7. Die Zahl der Einheiten in der ersten Metaphase variiert zwischen 29 (Abb. 3) und 34 (Abb. 5), wobei die grösste Häufigkeit des Vorkommens auf die Elemente 30–31 (Abb. 4) fällt. Wenn wir auf Grund vorhergegangenen Studiums annehmen, dass die Chromosomen des Haploids *N. rustica*, der uns zur Verfügung stand, nicht miteinander konjugieren, und wenn wir annehmen, dass bei *N. glauca* sich keine Autosyndese beobachten lässt, so kann die oben angeführte Zahl der Einheiten auf folgende Weise entziffert werden: die Chromosomen der 2 Genome von *N. glauca* konjugieren miteinander und ergeben Bivalente, die zum Teil mit den Chromosomen von *N. rustica* konjugieren, indem sie Trivalente bilden. Die übrigen Chromosomen von

¹⁾ Der Sesquidiploid (von sesqui = $1\frac{1}{2}$, WEBBER 1930) stellt einen Allopolyploid vor, welcher 2 Genome von der einen Elternform u. 1 Genom von der anderen Elternform aufweist

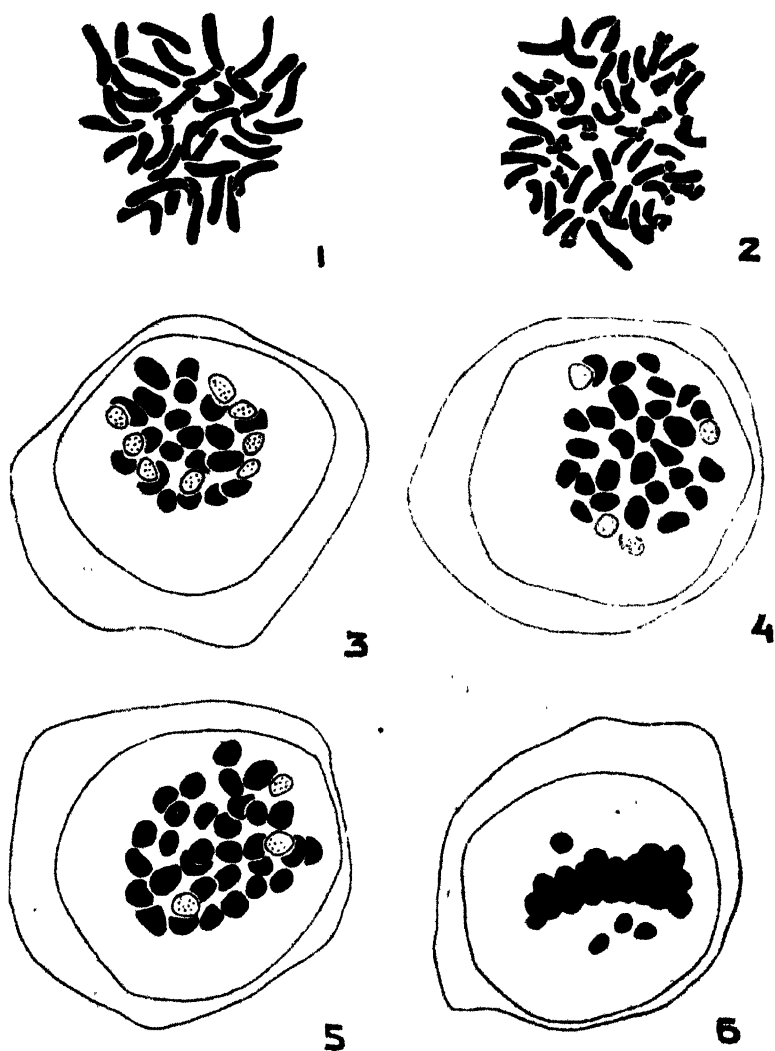


ABB. 1. Somatische Kernplatte aus dem Würzelchen eines digenomen Bastards $F_1 N. rustica \times N. glauca$ — 36 Chromosomen (vergr. 2300)

ABB. 2-6. Sesquidiploid $N. rustica \times N. glauca$:

ABB. 2. Somatische Kernplatte aus dem Wurzelchen eines Sesquidiploids — 48 Chromosomen (vergr. 1350); Abb. 3-5 Metaphasen der heterotypischen Teilung einer Pollen-Mutterzelle (P.M.Z.) vom Pol; 28, 31, 34 Einheiten (vergr. 2300); Abb. 6 — Metaphase der heterotypischen Teilung in Seitenansicht.

N. rustica bestehen weiter als Univalenten. Auf diese Weise gibt die bei dem Sesquidiploid festgestellte Anzahl von Einheiten (zur Untersuchung kam 1 Pflanze) folgende vorauszusetzende Konjugationstypen in der Metaphase der heterotypischen Teilung.

TAB. 2. TYPEN DER KONJUGATION IN DER METAPHASE DER HETEROTYPISCHEN TEILUNG BEI DEM SESQUIDIPLOID *N. rustica* \times *N. glauca*

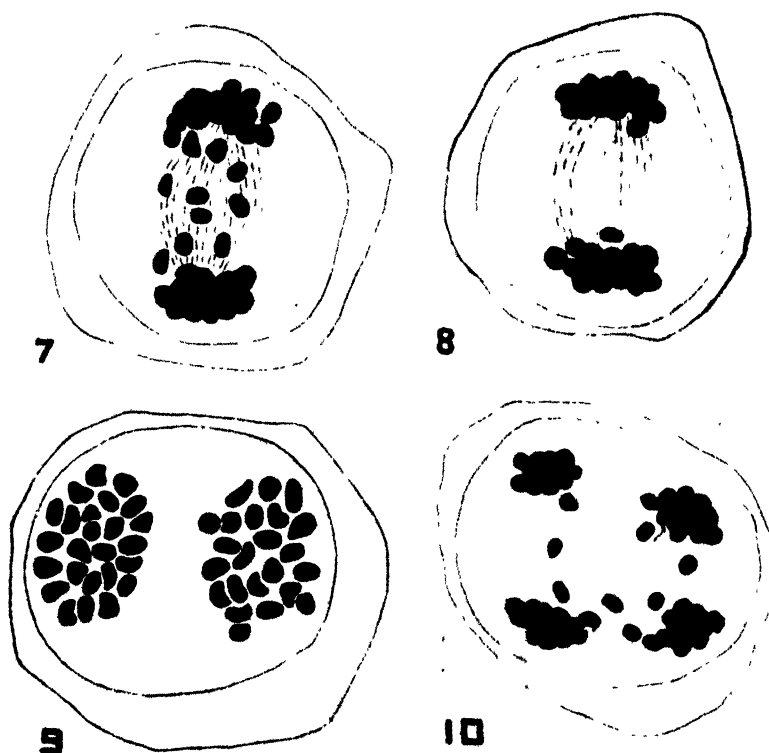
Anzahl der Einheiten	Typen der Konjugation.
28	8 III + 4 II + 16 I
30	6 III + 6 II + 18 I
31	5 III + 7 II + 19 I
32	4 III + 8 II + 20 I
33	3 III + 9 II + 21 I
34	2 III + 10 II + 22 I

Die Chromosomen der Metaphase der heterotypischen Teilung erscheinen als äusserst verschieden in Form und Grösse (Abb. 3–5). Morphologisch lässt sich manchmal das Vorhandensein von Trivalenten nachweisen.

Im Anfang der Teilung ist die heterotypische Anaphase deutlich unregelmässig (Abb. 7). Den Verlauf der Teilung müssen wir uns in folgender Weise vorstellen: die Bivalenten verteilen sich nach den Polen vollkommen ordnungsmässig; bei den Trivalenten gehen 2 Chromosomen zu dem einen Pol ab, der dritte aber zu dem andern, während die Univalenten zwischen den Polen wandern und sich zufällig verteilen. Teilungen von Univalenten wurden nicht beobachtet. Gegen Ende der Anaphase erreichen die zurückbleibenden Chromosomen gewöhnlich die Pole (Abb. 8). In der Interkinese kamen zuweilen Chromosomen zum Vorschein, die nicht in Kerne eingeschlossen waren. Manchmal wurden Figuren beobachtet, welche augenscheinlich für einkernige Interkinese gehalten werden müssen, die infolge eines Ausfallens der heterotypischen Teilung entstanden ist.

Die Metaphase der homöotypischen Teilung in solchen Fällen wo die Chromosomen genügend gut verteilt sind, ergibt in beiden Platten 48 Chromosomen (Abb. 9). Ihre Verteilung in den Platten ist infolge

der oben erwähnten Unregelmässigkeiten der heterotypischen Teilung nicht gleichmässig. Es wurden folgende Beziehungen festgestellt: 20 + 28, 21 + 27, 22 + 26, 23 + 25, 24 + 24. Nicht selten kamen Figuren mit einzelnen Chromosomen vor, welche in die Plat-



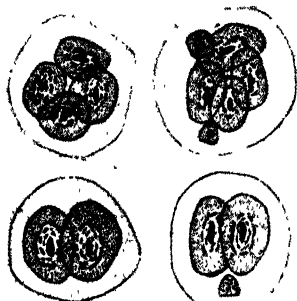
Sesquidiploid *N. rustica* × *N. glauca*

Abb. 7-8 Frühe und späte Anaphase der heterotypischen Teilung des P. M. Z. (vergr. 2300); Abb. 9 — Metaphase der homootypischen Teilung der P. M. Z. — 23 + 25 Chromosomen (vergr. 2300), Abb. 10 — Anaphase der homootypischen Teilung der P. M. Z. (vergr. 2300).

ten nicht eingeschlossen waren. Die Berechnungen waren in solchen Fällen durch Vorhandensein sekundärer Chromosomenassoziationen erschwert. In der Anaphase der zweiten Teilung kommen nicht selten zurückbleibende Chromosomen vor (Abb. 10).

Die Analyse des Sporenstadiums ist in Tab. 3 dargestellt. Die

allgemeine Unregelmässigkeit der Reduktionsteilung macht sich in entsprechender Weise bei den Sporaden geltend, indem sie zur Bildung einer bedeutenden Anzahl von Zellen mit Mikronuklei beiträgt. Gleichzeitig ist auch eine genügende Anzahl von normalen Tetraden vorhanden (Abb. 11). Auffallend ist die grosse Menge von Dyaden, was auf die Bildung von Gameten mit nicht reduzierter Chromosomenzahl hinweist. Wenn auch im weiblichen Gametophyt die Reduktionsteilung nach gleichem Typus verläuft (und wir haben keinen Grund diese Annahme zu verwerfen), so müssen bei der Bestäubung des Sesquidiploids der Mutterform *N. rustica* in der Nachkommenschaft Amphidiploiden $24 rr + 12 gg$ erhalten werden. Die Bestäubung der Vaterform aber kann tetragenome Formen mit einem Genom von *rustica* und 3 Genomen von *glauca* $24 r + 12 ggg$ ergeben.



H

Abb. 11 — Sporadentypen.

TAB. 3. ANALYSE DER SPORADEN BEI DEM SESQUIDIPLOID VOM JAHRE 1932

	Anzahl der Fälle	% %
Dyaden	31	7.7
Dyaden mit Micronukleus.	3	0.7
Triaden	1	0.2
Tetraden	113	77.5
Tetraden mit 1 Mikronukleus	52	12.9
Tetraden mit 2 Mikronuklei	4	1.0
	404	100.0

Der Pollen des Sesquidiploids ist in Grösse und Form nicht einheitlich (Abb. 12). Eine sehr grosse Anzahl von den Pollenkernen weisen keinen Inhalt auf. Die Menge normalen, ausgefüllten Pollens bei 3 untersuchten Pflanzen schwankt zwischen 4–18.

Infolge einer Reihe von Merkmalen (gestielte Blätter, Breite der Blüte u.s.w.) welche die gekreuzten Arten gemein haben, unterscheiden sich die Sesquidiploiden unbedeutend von gewöhnlichen Bast-

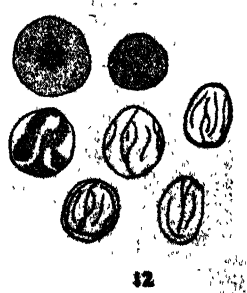


Abb 12 — Pollentypen

arden. Diese Unterschiede kommen in einer geringeren Behaarung, einer breiteren Blüte u.s.w. zum Vorschein. Bei freier Blüte fruchten sie reichlich; unter dem Isolator jedoch ist es uns trotz einer grossen Anzahl isolierter Blüten und künstlicher Bestäubung in all den Versuchsjahren nicht gelungen auch nur einen Samen zu erhalten. Sie müssen also als selbststeril angesehen werden. Rückkreuzungen mit der Vaterform d.h. mit *N. glauca* ergaben gute Resultate und eine grosse Anzahl vollkommene ausgebildeter

Pflanzen, Kreuzungen mit der Mutterform d.h. mit *N. rustica* gelingen mit grosser Mühe. Vollkommen günstig verlaufen Kreuzungen mit *N. tabacum* und einigen andern Arten.

Das Entstehen der gegebenen Sesquidiploidpflanzen muss durch das Vorhandensein eines verdoppelten Chromosomensatzes im männlichen Gamet von *N. glauca* erklärt werden. Die Fälle, wo Polyploiden von diesem Typus erhalten wurden, müssen als erste in der Literatur verzeichnet werden. GATES (1929) hält das Erhalten eines triploiden Mutanten durch Verschmelzung diploiden Pollens mit einem haploiden Ei für wenig wahrscheinlich. Wir nehmen an, dass bei uns gerade solch ein Fall zu stande kam und dass keine Erscheinung von Dispermie vorliegt. Einen Beweis dafür liefert eine ziemlich grosse Häufigkeit der Chromosomenverdoppelung in den Gameten von *N. glauca*, was aus den von uns ausgeführten Untersuchungen hervorgeht, welche den Zweck hatten, den Einfluss niedriger natürlicher Temperaturen auf die Bildung von Gameten mit verdoppeltem Chromosomensatz zu bestimmen. Die *Nicotiana* Arten verhalten sich verschieden zu der Einwirkung niedriger Temperaturen auf den Verlauf der Genogenese; so ergeben *N. Sanderac* und *N. glutinosa* überhaupt keine Dyaden. *N. sylvestris* ergab bei gleichen Bedingungen 2.8% Dyaden. Ganz überraschend war der Umstand, dass die Art *N. glauca* der winterfesteste Repräsentant dieser Gattung, eine ziemlich grosse Anzahl Dyaden lieferte. In einzelnen Prä-

paraten erreichte ihre Anzahl 13%, im Durchschnitt aber ergab die Berechnung von 1285 Zellen 8,0%. *N. glauca* offenbart also in dieser Hinsicht einen spezifischen Charakter (TERNOVSKY — 1935). Bei der gegebenen Art fiel es nicht schwer doppelten Chromosomensatz in den Mutterzellen des Pollens nachzuweisen (Abb. 13).

Die Verdoppelung findet hier augenscheinlich am häufigsten als Resultat des Ausfallens der heterotypischen Teilung statt. Das Auftreten von vier Sesquidiploiden *rustica* \times *glauca* mit zwei Genomen der Vaterpflanze halte ich daher durchaus nicht für überraschend. Die Zahl der Sesquidiploiden wäre zweifellos bedeutend grösser, wenn eine grössere Anzahl von Pflanzen zur Untersuchung käme.

Sowohl die Digenomen als auch die sesquidiploiden Bastarde *N. rustica* \times *glauca*, die seit 1931 von uns erhalten werden, erscheinen als die ersten gelungenen Bastarde dieser Kombination. Ihre Ausnutzung zu Zwecken der Pflanzenzüchtung bietet augenscheinlich ein grosses Interesse in Hinsicht des Erlangens solcher kräftiger Formen, denen im höher Gehalt an Citronensäure und eine Immunität gegen eine Reihe von Infektionskrankheiten eigen ist. Das Erlangen solcher Formen ist auf verschiedenem Wege möglich; durch das Auftreten von „cross-over“ den Ersatz eines Teiles der Chromosomen von *rustica* durch Chromosomen von *glauca* und das Erscheinen konstanter aneuploider Formen, welche einen vollzahligen Komplex von Chromosomen von *rustica* und eine mehr oder minder grosse Anzahl von gepaarten Chromosomen von *glauca* enthalten.

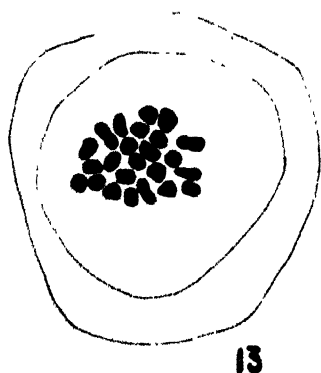


Abb 13 Metaphase, Pollen Mutterzelle von *N. glauca* mit doppeltem Chromosomensatz (acet-karmin-Preparat) (vergr 1600)

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DIE HEREDITÄT DES ALBINISMUS

(Aus dem niederländischen Institut für menschliche Erblchkeitsforschung und Rassenbiologie. Abteilung für medizinische statistische Erblchkeitsforschung, Direktor Dr. J. SANDERS)

VON

J. SANDERS

(Manuskript eingegangen am 16. Mai 1937)

Wir haben früher (Genetica XVI 1934 und XVII 1935) 3 Familien mit erblichem Albinismus circumscriptus beschrieben. Diese Art des Albinismus ist dominant und zeigt einen oder mehrere weissen Flecke auf der Haut oder im Kopfhaar. Beim Albinismus totalis — und darüber handelt es sich in dieser Publikation — sind die Haare weisz mit mehr oder weniger gelblichem Anstrich. Haut und Augen scheinen pigmentlos zu sein. Die klinischen Symptome des Albinismus (das Wort totalis lassen wir fortan fort) sind hauptsächlich nahezu pigmentlose Kopfhaare, Augenbrauen, Wimpern und Haut. Weiter häufig Strabismus, Refraktionsanomalien, stark durchleuchtbare Iris (bei dunklen Rassen viel weinger, da hier das Stroma iridis häufig stark entwickelt ist), Fehlen des Makulagelbs oder mangelhafte Entwicklung desselben, Nystagmus und Lichtscheu. Die Pupillen leuchten sehr oft rötlich auf. Die Albinos dunkeln nicht in der Sonne nach, sondern sie bekommen an den Stellen, die dem starken Sonnelicht kurze Zeit ausgesetzt waren, rote, schmerzhaft Brennblassen.

Es hat sich ergeben, dass beim Albinismus nicht alle Pigment abwesend ist. WAARDENBURG schreibt: „Es ist falsch, wenn man beim totalen Albinismus völlige Abwesenheit von Pigment annimmt. Spuren scheinen fast immer vorhanden zu sein; und jedenfalls dunkeln die anfangs albinotischen Individuen meistens später etwas nach“.

Was die Literatur betrifft, WAARDENBURG hat in seinem vor einigen Jahren erschienenen Buche „Das menschliche Auge und seine Erbanlagen“ (herausgegeben in 1932 von M. Nijhoff, Haag), eine sehr ausführliche Liste publiziert. Es ist deshalb unnötig dies hier noch einmal zu erwähnen.

Der Albinismus kommt in unserem Lande selten vor. Schon vor einigen Jahren hat WAARDENBURG etwa 40 Familien gefunden, wo einer oder mehrere der Geschwister Albinos waren. Eine allgemeine Nachforschung der Verbreitung dieser Abweichung hat bis jetzt aber noch nicht statt gefunden. Dazu haben wir ein Rundschreiben an alle Augenärzte geschickt und einen Aufruf in der niederländischen medizinischen Wochenschrift geschrieben. Die empfangenen Antworten haben zu dieser Publikation geführt.

WAARDENBURG unterscheidet folgende Typen:

1. *Albinismus universalis fere completus*, mit Unterentwicklung der Maculae luteae. Nystagmus ist sekundär. Diese Form ist autosomal-rezessiv.

2. *Albinoidismus sive Albinismus universalis incompletus*, ebenfalls mit Unterentwicklung der Makulae. Diese Form ist ebenfalls autosomal-rezessiv oder (seltener) unregelmässig-dominant. Nystagmus ist sekundär; er kann auch fehlen. Gelegentlich besteht auch Kopfwackeln.

3. *Isolierter Augen- bzw. Fundusalbinismus* mit Unterentwicklung der Makulae, sekundärem Nystagmus und fakultativem Kopfwackeln. Diese Formen sind rezessiv am X-Chromosom gebunden, gonosomal-rezessiv. Der Nystagmus kann beim Fundusalbinismus fehlen.

4. *Aplasia*, bzw. *Hypoplasia* Maculae luteae, gegebenenfalls höchstens mit Pigmentarmut des Augenhintergrundes und mit sekundärem Nystagmus verbunden. Diese Form ist gonosomal-rezessiv und autosomal-rezessiv, oder möglicherweise autosomal-unregelmässig-dominant. Zuweilen fehlt das Augenzittern.

In einer späteren Veröffentlichung (Vererbungsergebnisse und -probleme am menschlichen Auge; Referat auf der Tagung in Jena, Juli 1935. Zeitschrift für induktive Abstammungs- und Vererbungslehre B. 70, Heft 3/4) hat WAARDENBURG die Möglichkeit erwähnt, dass es sich bei den kompletten bzw. inkompletten Formen je um Polyallelie handelt.

Wir haben die Augenärzte gefragt die Fälle bei einer der drei folgende Gruppen einzuteilen:

A. *Albinismus universalis fere completus*. Symptome: Foveahypoplasie bzw. -aplasie, Nystagmus, stark diaphane Iris. Pigment fehlt fast ganz in Haut, Haaren und Augen.

B. *Albinismus universalis incompletus* (Albinoidismus) von Haut, Haaren und Augen; aber im späteren Alter erscheint doch etwas Pigment, vielfach auch im Irisstroma, und ein wenig im Irisepithel. Diaphane Iris, Nystagmus (häufig) und Kopfwackeln (selten), Foveahypoplasie bzw. -aplasie.

C. *Albinismus nur vom Auge oder Fundus mit Foveahypoplasie* bzw. -aplasie, Nystagmus (häufig), Kopfwackeln (häufig).

Weiter wurde gefragt, ob es familiär war, ob die Eltern blutverwandt waren, und ob die Eltern oder einer von ihnen leichte Symptome zeigten wie Pigmentarmut oder diaphane Iris, wie sie von WAARDENBURG gefunden wurde, u.s.w.

Leider waren die Antworten nicht alle so ausführlich wie erwünscht war, sodass die Fälle nicht in den drei Gruppen eingeteilt werden konnten. So weit es möglich war, haben wir noch nachgefragt oder selbst versucht die Patienten zu untersuchen, oder vom Spezialisten untersuchen zu lassen.

Der Erfolg dieses Rundschreibens war, dass uns 140 Familien mit 216 Albinos gemeldet wurden. Hier folgt eine Liste aller Fälle mit Allem, was wir davon bis jetzt wissen.

Zuvor sei bemerkt, dass 5 Fälle gemeldet sind, deren Eltern Ausländer waren. Diese Fälle sind nicht in den 140 einbegriffen, weil wir keine genealogischen oder erblichen Nachforschungen hierüber einstellen konnten.

Von den 140 Fällen wurden 37 schon von WAARDENBURG untersucht. Sie sind aber nicht genau genealogisch durchforscht, sodass wir sie jetzt nochmals untersuchten, in der Hoffnung dabei gemeinsame Ahnen bzw. Stammeltern herauszufinden.

In verschiedenen Fällen war es nicht möglich die Ahnentafel zusammenzustellen. Bisweilen waren die nötigen Kirchbücher nicht anwesend, oder scheiterte die Nachforschung, weil der Geburtsort und das Datum nicht zu finden waren. In anderen Fällen war der Probandus oder einer der Vorfahren ein uneheliches Kind. Eine grosse Lücke in den genealogischen Nachforschungen ist dadurch

entstanden, dass wir in 3 Provinzen keine Hilfe hatten für die Prüfung der Kirchbücher, die in den Reichs- und Gemeindearchiven anwesend sind, sodass verschiedene Familienzweige nicht weiter als bis etwa 1775 nachgewiesen werden konnten. Aus finanziellen Gründen war es nicht möglich für diese Arbeit bezahlte Kräfte zu nehmen.

In der nun folgenden Liste sind zuerst die Albinos genannt; dann folgen die Zahlen aller Knaben und Mädchen in der Familie und dann die Geburtenfolgennummer der Albinos. Mit Blutverwandtschaft ist immer gemeint die Verwandtschaft zwischen den Eltern; mit Verwandtschaft die Familienverwandtschaft mit andern Albinos.

Liste der Fälle

K = Knabe, M = Mädchen. G = Geburtenfolgennummer, B = Blutverwandtschaft

1. Albinos 1 K und 1 M. Total 6 K und 2 M. G 5 und 6. Die Eltern haben sehr dunkles Haar.

2. Albino 1 K. Total 2 K. G 1. B 6e Stufe.

3. Albinos 1 K und 3 M. Total 8 K und 5 M. G 4, 5, 9, 10. Der männliche Probandus hat selbst 2 K und 1 M, die normal sind. Verwandtschaft mit 113 (Fig. 1). Vatersbruder und Vatersschwester sind geisteskrank. Bruder war blind geboren und hatte Hydrocephalus; ist verstorben. Vatersmutter Alkoholiker. Großeltern Vatersseite sind Alkoholikerkinder.

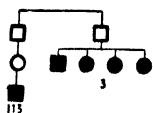


FIG. 1.

4. Albinos 2 K und 1 M. Total 4 K und 4 M. G 2, 3, 6.

5. Albino 1 K. Total 4 K und 2 M. G 1. B 6e Stufe.

6. Albino 1 K. Total 2 K und 1 M. G 1. Verwandtschaft mit 14, 52 und 53 (Fig. 2).

7. Albino 1 K. Total 2 K und 4 M. G 3. Verwandtschaft mit 4 (Fig. 3). Alle Kinder mit Ausnahme des Albinos haben sehr dunkles Haar. Patient hat selbst 2 K und 1 M, die sehr dunkles Haar haben.

8. Albinos 1 K und 1 M. Total 2 K und 2 M. G 1 und 4. Ahnentafel mit Lücken.

9. Albino 1 K. Total 2 K und 3 M. G 3. B zweimal 6e Stufe, 8e Stufe, 10e Stufe (Fig. 4). Verwandtschaft mit 29 (Fig. 5).

10. Albino 1 M. Total 1 K und 1 M. G 2.

11. Albino 1 M. Total 4 K und 7 M. G 3. B 4e Stufe.

12. Albino 1 M. Total 2 M. G 2. Verwandtschaft mit 20 (Fig. 6). Ahnentafel mit Lücken. Vatersvater war ein uneheliches Kind. Probandus hat Kopfsaar, Augenbrauen und Wimpern sehr blond.

Kein Kopfwackeln oder Nystagmus. Irisstroma blau; Iris nicht diaphan. Fundi pigmentlos; nur im rechten Fundus kleiner Papillarsaum; peripapilläre, myopische Degeneration. Refraktion Rechts

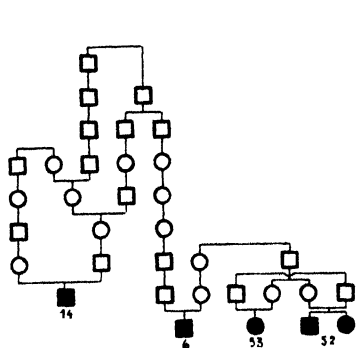


FIG. 2.

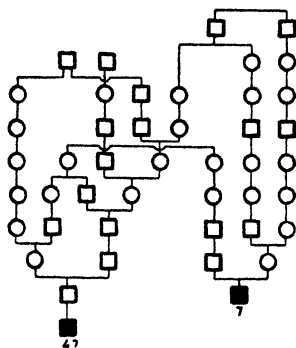


FIG. 3.

Astigmie E + 18 und E + 16, links Astigmie E + 14,5 und E + 11. Visus rechts nach Korrektur $1/3$, links nach Korrektur $1/2$.

13. Albino 1 M. Total 2 K und 2 M. G 1. B zweimal 10e Stufe (Fig. 7).

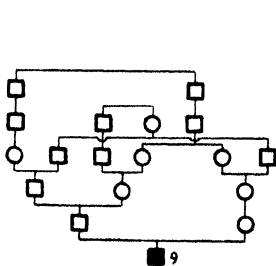


FIG. 4.

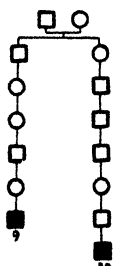


FIG. 5.

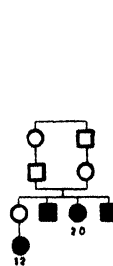


FIG. 6.

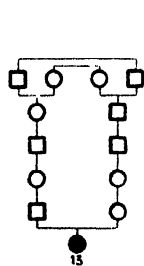


FIG. 7.

14. Albino 1 K. Total 1 K und 1 M. G 2. B 8e Stufe. Verwandtschaft mit 6 (Fig. 2).

15. Albino 1 M. Total 2 K und 4 M. G 4. Ahnentafel mit Lücken.

16. Albinos 2 K und 1 M. Total 2 K und 3 M. G 2, 3 und 4. Ahnentafel mit grossen Lücken.

17. Albinos 1 K und 1 M. Total 1 K und 3 M. G 1 und 4.

18. Albino 1 K. Total 1 K und 1 M. G 1. B 4e Stufe.

19. Albinos 3 K. Total 5 K und 2 M. G 2, 4 und 5. Ältester Albino hat Kopfhaar, Augenbrauen und Wimpern völlig pigmentlos; kein Kopfwackeln; Strabismus divergens oculis sinistri; doppelseitiger Nystagmus horizontalis; Irisstroma blau; Iris stark diaphan; Fundi

oculi pigmentlos, Papille normal, sehr lichtscheu, Refraktion an beiden Seiten E = -3. Acies Visus rechts $\frac{1}{10}$, nach Korrektion etwas mehr, links $\frac{1}{10}$, nicht zu korrigieren.

Der dritte Albino hat Kopflhaar, Augenbrauen und Wimpern weiss, doppelseitige Nystagnus horizontalis; blaue Iris Acies Visus beiderseitig $\frac{5}{20}$. Eltern und Geschwister dunkles Haar

20. Albinos 2 K und 1 M. Total 5 K und 4 M. G 2, 5 und 9. B 4c Stufe. Verwandtschaft mit 12 (Fig. 6). Weibliche Albino hat Kopflhaar sehr blond-grau Augenbrauen und Wimpern pigmentlos. Kein Kopfwackeln. Stand der Augen recht. Beiderseits langzamer, horizontaler Nystagnus. Irisstroma grau-blau mit gelbgrauen Pigmentstuckchen. Papillensaum am Irishinterblatt fehlt, Iris ganz diaphan. Fundi pigmentlos. Grosse myopische Sehiel. Recht und links Refraktion E = -17. Visus rechts $\frac{3}{60}$, nach Korrektion $\frac{1}{10}$; links $\frac{3}{60}$, nach Korrektion $\frac{1}{10}$.

Altester, männlicher Albino hat Kopflhaar gelb. Augenbrauen und Wimpern pigmentlos. Kein Kopfwackeln, kein Strabismus, langsamer, beiderseitiger, horizontaler Nystagnus. Irisstroma grau mit gelbbraunen Pigmentkornchen. Am Irishinterblatt fehlt der Pupillarsaum, ganz diaphaner Iris, Fundi beiderseits pigmentlos. Foveahypoplasie. Refraktion rechts und links astigmatisch E = -4 und E = -3. Visus beiderseits $\frac{1}{10}$, keine Korrektion. Ahnen haben, soweit bekannt, dunkles Haar.

21. Albinos 2 K. Total 6 K und 3 M. G 2 und 3

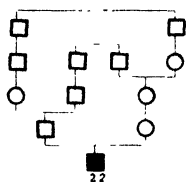


FIG. 6

22. Albino 1 K. Total 6 K und 4 M. G 6. B 6c und 8c Stufe (Fig. 8).

23. Albinos 1 K und 1 M. Total 2 K und 4 M. G 3 und 5. Männlicher Albino hat pigmentloses Kopflhaar, und auch Augenbrauen und Wimpern pigmentlos. Kopf nach rechts gedreht. Kein Kopfwackeln, Nystagnus horizontalis. Irisstroma blau mit gelbem Pigment. Irishinterblatt diaphan; Pupillarsaum anwesend; Fundi pigmentlos, Maculahypoplasie; kein Reflexring. Refraktion rechts und links E = -4. Visus rechts $\frac{1}{6}$, nach Korrektion $\frac{1}{4}$; links $\frac{1}{10}$, nach Korrektion $\frac{1}{6}$.

Weibliche Albino hat gelbes Kopflhaar, Augenbrauen und Wimpern pigmentlos. Kopfhaltung recht; kein Wackeln. Nystagnus horizontalis; Irisstroma blau, pigmentlos, Irishinterblatt ganz diaphan. Pupillarsaum anwesend; Fundi pigmentlos; Foveaaplasie! Refraktion rechts und links astigmatisch E = -4 und E = -2. Visus rechts und links $\frac{5}{60}$.

Vater ist taubstumm.

24. Albino 1 M. Total 3 K und 6 M. G 8.

25. Albino 1 K. Total 3 K und 2 M. G 3. Verwandtschaft mit 70 (Fig. 9). Kopflhaar, Augenbrauen und Wimpern blond. Beiderseits rotatorer Nystagnus. Irisstroma blau; Pupillarsaum anwesend;

schmaler peripherer Instand diaphan, Foveahypoplasie; Fundus pigmentlos, nur in der Peripherie Chorioidtissnarben mit sehr wenig Pigment. An Papillen myopische Sicel, am Macula kein Reflexring

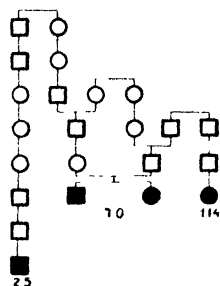


FIG. 9

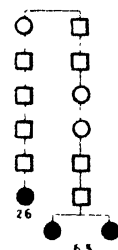


FIG. 10

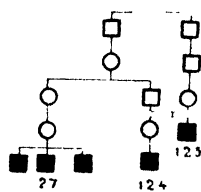


FIG. 11

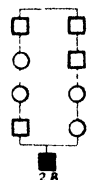


FIG. 12

oder Maculagelb. Refraktion rechts $E = +10$, links $E = +8$ Visus rechts $2'_{200}$, nach Korrektur $1'_{10}$, links $4'_{60}$, nach Korrektur $1'_{4}$

26. Albino 1 K. Total 2 K und 2 M. G 1. Verwandtschaft mit 63 (Fig. 10).

27. Albinos 3 K. Total 11 K und 4 M. G 1, 5 und 6. Verwandtschaft mit 124 und 125 (Fig. 11). Weil von 124 die Mutter und die Schwester Nystagmus haben, ist die Frage, ob die Pigmentarmut hier Zufall ist, und ob hier nicht unregelmässig-dominanter hereditärer Nystagmus besteht. Der Augenarzt hat hier doch bestimmt Albinismus angenommen.

28. Albino 1 K. Total 4 K und 1 M. G 4. B 8e Stufe (Fig. 12).

29. Albino 1 K. Total 3 K. G 1. Verwandtschaft mit 9 (Fig. 5).

30. Albinos 2 K und 1 M. Total 4 K und 4 M. G 1, 5 und 8. Ahnentafel mit Lücken.

31. Albino 1 M. Total 1 K und 3 M. G 3. Verwandtschaft mit 32 (Fig. 13).

32. Albinos 1 K und 1 M. Total 1 K und 4 M. G 2 und 5. Verwandtschaft mit 31 (Fig. 13).

33. Albinos 1 K und 1 M. Total 2 K und 1 M. G 2 und 3. B 4. Ahnentafel mit vielen Lücken.

34. Albino 1 M. Total 3 M. G 2. Ahnentafel mit grossen Lücken.

35. Albinos 2 M. Total 2 K und 3 M. G 1 und 5. B 6e Stufe.

36. Albino 1 K. Total 5 K. G 4. Ahnentafel mit grossen Lücken.

37. Albino 1 M. Total 1 K und 1 M. G 2. Verwandtschaft mit 130 (Fig. 14).

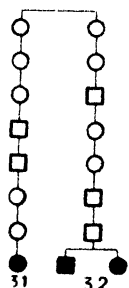


FIG. 13

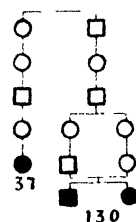


FIG. 14

38. Albinos 2 K. Total 5 K und 3 M. G 4 und 6. Ahnentafel mit vielen Lücken.

39. Albino 1 M. Total 2 K und 1 M. G 3.

40. Albinos 2 K. Total 3 K und 2 M. G 3 und 5. B 4e Stufe. Verwandtschaft mit 54 (Fig. 15).

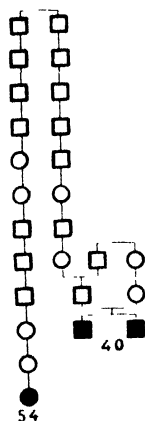


FIG. 15

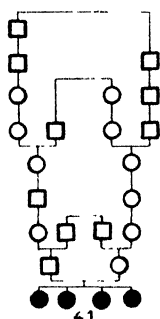


FIG. 16

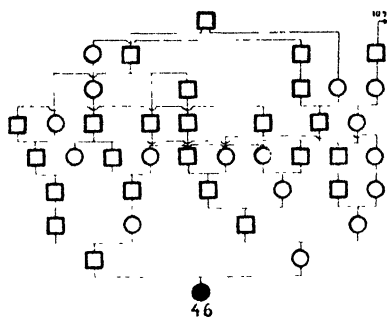


FIG. 17

41. Albinos 4 M. Total 4 K und 4 M. G 2, 4, 6, und 8. B 4e, 12e und 13e Stufe. (Fig. 16).

42. Albino 1 K. Total 1 K. G 1. B 4e Stufe

43. Albino 1 K. Total 4 K und 2 M. G 6. Hat selbst eine normale Tochter.

44. Albino 1 K. Total 3 K und 3 M. G 1.

45. Albino 1 M. Total 2 K und 4 M. G 6

46. Albino 1 M. Total 1 K und 1 M. G 1. B 8e dreimal, 10e 5 mal, 12e 2 mal und 14e Stufe (Fig. 17).

47. Albino 1 K. Total 1 K. G 1. Verwandtschaft mit 7 (Fig. 3).

48. Albino 1 M. Total 2 K und 1 M. G 1. Verwandtschaft mit 49 und 111 (Fig. 18). Probandus hat 5 normale Kinder.

49. Albinos 5 K. Total 8 K und 2 M. G 2, 7, 8, 9 und 10. Verwandtschaft mit 48 und 111 (Fig. 18). Von diesen 5 Albinos sind 2 vom Augenarzt noch einmal untersucht worden. Sie haben fast völlig pigmentlose Haare, Nystagmus, sehen sehr schlecht, tragen dunkle Brillengläser. Weil hier Totalalbinismus vorliegt, müssen die Eltern heterozygot sein. Es ist wohl ein grosser Zufall, dass die Tochter eines dieser Albinos wieder ein Heterozygot geheiratet hat. Dieser Mann stammt aus Konstanz. Von ihm ist nichts weiter zu erfahren.

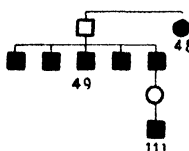


FIG. 18.

50. Albino 1 K. Total 1 K und 1 M. G 2. Ahnentafel mit Lücken.
 51. Albino 1 K. Total 1 K und 2 M. G 1. Muttersvatersvater war wahrscheinlich auch Albino. Er wurde „Der Weisse“ genannt, und in der Familie ist er noch so bekannt.

52. Albinos 1 K und 1 M. Total 1 K und 3 M. G 2 und 3. Verwandtschaft mit 6 und 53 (Fig. 2).

53. Albino 1 M. Total 1 K und 2 M. G 1. Verwandtschaft mit 6 und 52 (Fig. 2).

54. Albino 1 M. Total 1 M. G 1. Verwandtschaft mit 40 (Fig. 15)

55. Albino 1 K. Total 4 K und 4 M. G 2. Ahnentafel mit Lücken.

56. Albinos 1 K und 2 M. Total 2 K und 6 M. G 1, 3 und 6. Alle drei Albinos haben Kopflhaare, Augenbrauen und Wimpern pigmentlos, Nystagmus, Iris diaphan, Fundi oculi pigmentlos. Ahnentafel mit Lücken

57. Albino 1 K. Total 2 K und 1 M. G 2

58. Albino 1 K. Total 2 K und 3 M. G 1. Ahnentafel mit vielen Lücken.

59. Albino 1 K. Total 2 K. G 2

60. Albino 1 M. Total 2 K und 4 M. G 5. B 8c Stufe (Fig. 19)

61. Albinos 1 K und 1 M. Total 1 K und 1 M. G 1 und 2

62. Albino 1 K. Total 4 K und 3 M. G 5. B 5c Stufe (Fig. 20). Ahnentafel mit Lücken.

63. Albinos 2 M. Total 2 K und 5 M. G 2 und 5. Verwandtschaft mit 26 (Fig. 10).

64. Albino 1 M. Total 1 K und 2 M. G 1. Ahnentafel mit vielen Lücken.

65. Albinos 3 M. Total 4 M. G 1, 2 und 3. Verwandtschaft mit 66

66. Albino 1 M. Total 4 K und 3 M. G 4. Dieser Albino ist die Mutter der 3 Albinos von 65. Ahnentafel lückenhaft. Dieser Albino hat wohl Iris und Sclera diaphan, aber keinen Nystagmus.

67. Albino 1 K. Total 3 K. G 2. Ahnentafel mit sehr grossen Lücken.

69. Albino 1 K. Total 1 K. G 1. B 4e Stufe.

70. Albinos 1 K und 1 M. Total 3 K und 2 M. G 3 und 4. Weibliche Albino hat albinotische Augen, Haare und Wimpern haben Silberfarbe, keinen Nystagmus, Lichtscheu. Der männliche Albino hat dieselbe Symptome, aber die Haare weiss. B 6e Stufe. Verwandtschaft mit 25 und 114 (Fig. 9)

71. Albinos 1 K und 2 M. Total 3 K und 4 M. G 2, 3 und 7.

72. Albinos 1 K und 2 M. Total 5 K und 5 M. G 5, 7 und 9. Ahnentafel mit Lücken.

73. Albino 1 K. Total 7 K und 3 M. G 2. B 4e Stufe. Ein Bruder ist blind geboren.

74. Albino 1 K. Total 4 K und 1 M. G 4. B 4e Stufe.

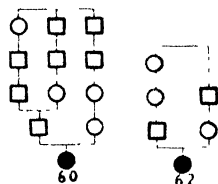


Fig. 19

Fig. 20

75. Albinos 1 K und 1 M. Total 2 K und 2 M. G 1 und 2. Ahnentafel mit Lucken.

76. Albino 1 M. Total 2 K und 1 M. G 2. Kopfhaar, Augenbrauen und Wimpern pigmentlos. Kein Kopfwackeln. Strabismus divergens Oculi sinistri. Nystagnus horizontalis. Irisstroma hellblau, pigmentlos. Iris diaphan. Pupillarsaum abwesend. Fundi oculi pigmentlos. Papillen normal; Refraktion rechts und links E 2. Visus rechts und links $\frac{1}{10}$. Geschwister und Eltern normal.

77. Albinos 1 K und 1 M. Total 2 K und 2 M. G 1 und 4. Mannerlicher Albino hat Epilepsie. B 4e, 6e 2 mal, 8e und 10e Stufe (Fig. 21)

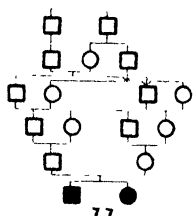


FIG. 21

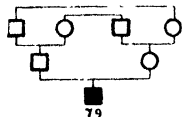


FIG. 22

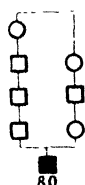


FIG. 23

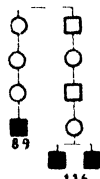


FIG. 24

78. Albino 1 M. Total 3 K und 5 M. G 2. B 4e Stufe. Ahnentafel mit Lucken.

79. Albino 1 K. Total 2 K und 1 M. G 1. B 4e Stufe 2 mal (Fig. 22)

80. Albino 1 K. Total 1 K und 1 M. G 2. B 7e Stufe (Fig. 23).

81. Albino 1 K. Total 1 K. G 1.

82. Albino 1 K. Total 1 K und 3 M. G 3. B 8e Stufe.

83. Albino 1 M. Total 3 K und 2 M. G 4. Ahnentafel mit Lucken.

84. Albino 1 K. Total 7 K. G 4. Ahnentafel mit Lucken.

85. Albino 1 M. Total 1 K und 2 M. G 2. Ahnentafel mit grossen Lucken.

86. Albino 1 K. Total 2 K und 3 M. G 2. Ahnentafel mit Lucken.

87. Albino 1 K. Total 3 K. G 2.

88. Albino 1. Total 8 K. G 8. War früher pigmentlos, jetzt dunkler geworden. Nystagnus. Kann in der Sonne nicht braun werden. Fundi oculi pigmentlos.

89. Albino 1 K. Total 2 K und 2 M. G 3. Verwandtschaft mit 116 (Fig. 24).

90. Albinos 3 K. Total 6 K und 3 M. G 3, 4 und 9. B 4e Stufe. Zwei dieser Albinos sind verstorben. Der dritte hat Kopfhaar, Augenbrauen und Wimpern pigmentlos, Kopfhaltung recht, Nystagnus in allen Richtungen; Irisstroma blau; Irishinterblatt ohne Pupillarsaum und ganz diaphan, Fundi oculi pigmentlos, vollkommen Macula-aplasie; Refraktion rechts und links E 8; Visus rechts und links $\frac{1}{60}$, nach Korrektion $\frac{5}{60}$.

91. Albinos 1 K und 1 M. Total 3 K und 3 M. G 3 und 4. Ahnentafel mit Lücken.

92. Albinos 1 K und 1 M. Total 2 K und 1 M. G 1 und 3. Verwandtschaft mit 107 (Fig. 25). Bei beiden Kopfhaar, Augenbrauen und Wimpern pigmentlos, kein Nystagmus, können in der Sonne nicht braun werden.

93. Albino 1 M. Total 1 K und 2 M. G 3. Ahnentafel mit Lücken.

94. Albinos 2 M. Total 4 M. G 1 und 2.

95. Albino 1 K. Total 2 K und 1 M. G 1. Der Bruder des Probandus hat eine weisse Haarlocke auf der rechten Kopfschale vom Geburt an, und hat ein Kind, das, wie er und seine Frau mir erzählt haben (ich habe es selbst leider nicht sehen können), völlig pigmentlose Kopfhare, Augenbrauen und Wimpern und Nystagmus hat. Er kann in der Sonne nicht braun werden und ist lichtscheu. Die Frau dieses

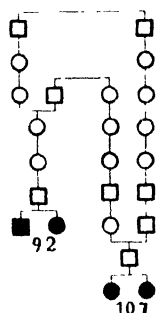


Fig. 25

Bruders hat eine Schwester, die vom Geburt an rechts auf dem Hinterkopf eine weisse Haarlocke hat. Diese zwei Schwestern haben eine

Kousine 2er Stufe (Fig. 26), die verheiratet ist mit einem Albino. Auch diesen habe ich nicht gesehen, weil diese Familie in Indien ist. Aber die Mutter, die sehr intelligent ist und gerade aus Indien kam, hat mich versichert, dass ihr Schwagersohn Albino ist, und einen Sohn hat der ebenfalls Albino ist. (Der Albinismus circumscriptus der zwei Personen ist in Fig. 26

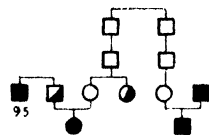


Fig. 26

angegeben durch schwarz machen der Hälfte)

96. Albino 1 M. Total 4 K und 6 M. G 10. Ahnentafel mit grossen Lücken.

97. Albinos 1 K und 1 M. Total 2 K und 2 M. G 3 und 4.

98. Albino 1 M. Total 1 K und 1 M. G 1. B 6e Stufe.

99. Albino 1 M. Total 3 M. G 2. Kopfhaar jetzt sehr blond, Augenbrauen fast pigmentlos und Wimpern blond, Kopf- und Augenstand normal, kein Nystagmus, Irisstroma blau, Iriskringen anwesend, Iris nicht diaphan, Fundus rechts und links pigmentlos, mit Ausnahme eines einzelnen Körnchen dem Papillenrand entlang, Papillen und Maculae normal, Refraktion Astigmatismus rechts und links $E: -3\frac{1}{2}$ horizontal, Visus rechts und links $\frac{1}{6}$, nach Korrektur $\frac{3}{4}$. Also ein Fall von Albinismus posterior. Eine Kousine sollte auch Albino sein. Sie hat ganz weisse Haare, ist nicht von mir untersucht worden. Ubrige Familienmitglieder normal.

100. Albino 1 M. Total 1 K und 1 M. G 1.

101. Albino 1 K. Total 1 K und 1 M. G 1. Ahnentafel mit grossen Lücken.

102. Albinos 2 M. Total 4 M. G 2 und 3.

103. Albinos 2 K. Total 4 K und 2 M. G 3 und 4. B 6e Stufe und viele höhere (Fig. 27) Verwandtschaft mit 107 und 140 (Fig. 38).

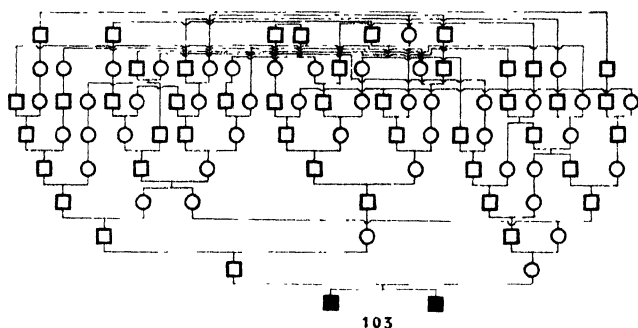


FIG. 27

104. Albinos 3 M. Total 1 K und 4 M. G 1, 2 und 5. Verwandtschaft mit 105 (Fig. 28).

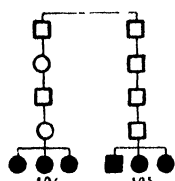


FIG. 28

105. Albinos 1 K und 2 M. Total 4 K und 3 M. G 1, 3 und 5. Verwandtschaft mit 104 (Fig. 28).

106. Albino 1 M. Total 1 K und 1 M. G 1.

107. Albinos 2 M. Total 4 K und 6 M. G 5 und 6. B verschiedene hohe Stufen (Fig. 29) Verwandtschaft mit 46, 92, 103, 112 und 140 (Fig. 30 und Fig. 39).

108. Albino 1 M. Total 1 K und 3 M. G 1. Ahnentafel mit Lücken

109. Albino 1 M. Total 4 K und 1 M. G 2

110. Albinos 2 K und 1 M. Total 5 K und 6 M. G 4, 8 und 9

111. Albino 1 K. Total 3 K. G 2. Verwandtschaft mit 49 (Fig. 18)

112. Albino 1 M. Total 4 K und 4 M. G 7. Verwandtschaft mit 107 (Fig. 30).

113. Albino 1 K. Total 1 K. G 1. Verwandtschaft mit 3 (Fig. 1). Probandus ist hellblond, Haut pigmentlos, Nystagmus rotatorius, Fundi oculi pigmentlos; Visus rechts $\frac{5}{50}$, links $\frac{5}{15}$.

114. Albino 1 M. Total 1 K und 5 M. G 1. Verwandtschaft mit 25 und 70 (Fig. 9). Ahnentafel mit Lücken.

115. Albino 1 K. Total 1 K. G 1.

116. Albinos 2 K. Total 2 K. G 1 und 2. Verwandtschaft mit 89 (Fig. 24). Ältester Albino hat hellblondes Kopfhair, Augenbrauen pigmentlos, hellblonde Wimpern, nystagmoide Bewegungen, Iris blau, normaler Struktur, Pupillarsaum anwesend, Iris nicht diaphan; Fundi oculi

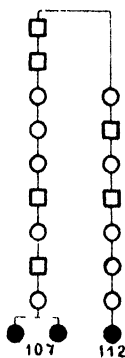


FIG. 30

pigmentlos, Papillen temporal etwas blass, Maculae normal, Refraktion rechts und links $E + 1\frac{1}{2}$. Patient ist imbecill, er spricht nicht, lacht nicht und ist taub. Der jüngste Albino hat Kopfhaar, Augenbrauen und Wimpern pigmentlos, keinen Nystagmus, Irisstroma blau, Iris nicht diaphan.

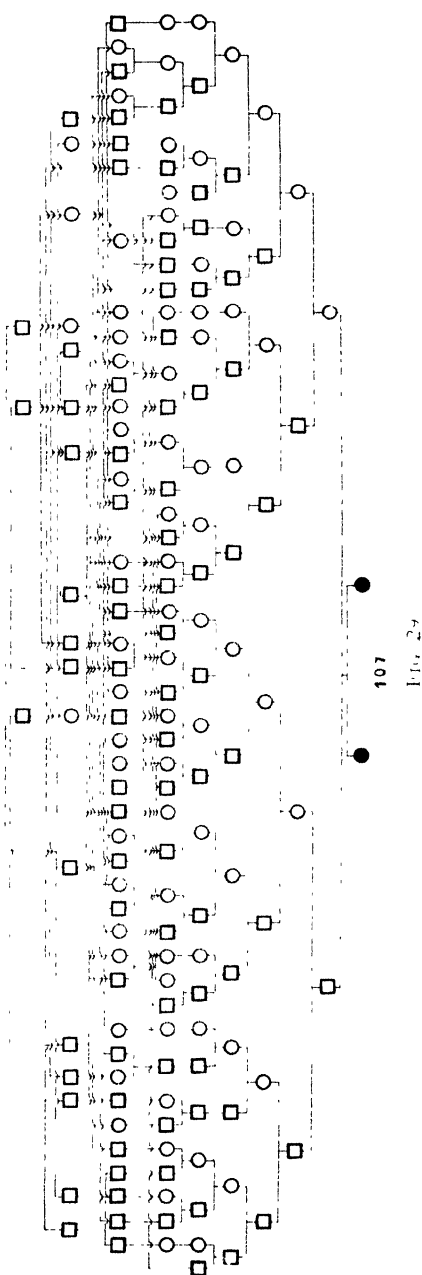
Verschiedene Familienmitglieder sind untersucht worden, aber normal gefunden. Nur von der Mutter wird erzählt, dass sie früher weisses Haar hatte, jetzt ist sie blond und weiter normal.

117. Albino 1 K und 1 M. Total 3 K und 1 M. G 2 und 3. Weibliche Albino hat goldfarbenes Kopfhaar, Augenbrauen und Wimpern hellblond, keinen Nystagmus, Irisstroma blau, Iris nicht diaphan, Pupilla-saum nicht anwesend, Fundi oculi pigmentlos. Papillen und Maculae normal.

Männlicher Albino ist pigmentlos, Nystagmus horizontalis, Myopia gravior $E + 10$. Familienmitglieder normal, einige haben wohl blondes Haar, aber keine Augenabweichungen.

118. Albino 1 M. Total 2 K und 1 M. G 1. Ahnentafel mit Lücken.

119. Albino 1 K. Total 3 K und 4 M. G 7. Verwandtschaft mit 120 (Fig. 31). Kopfhaar fast pigmentlos, Augenbrauen und Wimpern hell blond, kein Nystag-



mus, Irisstroma blau, Iris nicht diaphan, Fundi oculi pigmentlos, Papillen und Maculae normal.

120 Albino 1 K und 1 M. Total 1 K und 1 M. G 1 und 2 Verwandtschaft mit 119 (Fig. 31). Beide Albinos Kopflhaar, Augenbrauen und Wimpern hellblond, kein Nystagmus, Irisstroma grau blau, Iris nicht diaphan, Fundi oculi pigmentlos, Papillen und Maculae normal.

121 Albino 1 M. Total 1 K und 1 M. G 2. Verwandtschaft mit 134 (Fig. 32). Kopflhaare, Augenbrauen und Wimpern pigmentlos, Nei-

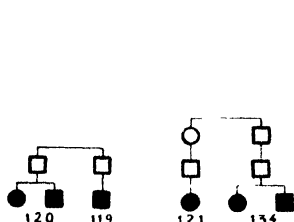


FIG. 31.

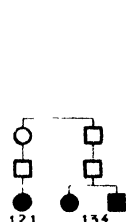


FIG. 32

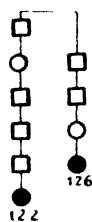


FIG. 33

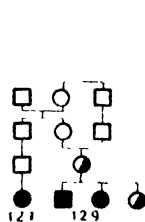


FIG. 34

gung nach rechts zu sehen, starker Nystagmus, Irisstroma blau, Iris stark diaphan, Fundi oculi pigmentlos, Refraktion rechts und links E 4.

122 Albino 1 M. Total 1 K und 2 M. G 1 Verwandtschaft mit 126 (Fig. 33). Kopflhaar, Augenbrauen und Wimpern hellblond, nystagmoide Bewegungen, Irisstroma blau und pigmentlos, Iris diaphan ohne Pupillarsaum, Fundi oculi pigmentlos. Schwester hat Katarakt. Weiter keine Besonderheiten in der Familie.

123. Albino 1 M. Total 1 K und 1 M. G 1. Hellblondes Haar, Nystagmus, Fundi oculi pigmentlos, lichtscheu, Haut pigmentlos, kann in der Sonne nicht nachdunkeln.

124. Albino 1 K. Total 5 K und 3 M. G 2. Verwandtschaft mit 27 und 125 (Fig. 11). Hellblondes Haar, Nystagmus, Fundi oculi pigmentlos. Die Mutter und eine Schwester haben Nystagmus beiderseits. Muttersbruder ist 125.

125. Albino 1 K. Total 2 K und 4 M. G 3. Verwandtschaft mit 27 und 124 (Fig. 11). Kopflhaar pigmentlos, Nystagmus, Fundi oculi pigmentlos, Probandus hat auch Epilepsie.

126. Albino 1 M. Total 3 K und 5 M. G 4. Ist von einem zweieiigen, gleichgeschlechtlichen Zwillings (diskordant). Verwandtschaft mit 122 (Fig. 33).

127. Albino 1 M. Total 1 K und 4 M. G 5. Verwandtschaft mit 129 (Fig. 34).

128. Albino 1 K. Total 5 K und 1 M. G 3. Kopflhaar pigmentlos, Nystagmus, lichtscheu, Strabismus convergens, Hypermetropie Rechts E + 2, links E + 1. Fundi oculi pigmentlos.

129. Albino 1 K und 1 M Total 2 K und 3 M, G 3 und 4 B 6e Stufe Verwandtschaft mit 127 (Fig. 34) Weibliche Albino ist von einem zweieigenen, gleichgeschlechtlichen Zwilling (diskordant). Weil diese Familie jetzt in Kalifornien wohnt, war Herr PAUL POPSON in Pasadena so liebenswürdig diese Albinos für mich zu untersuchen. Er schreibt mir folgendes:

„I compared the eyes with SALLER's Augenfarbentafel

Father Fairly dark brown hair (now shot with gray), very ruddy complexion Blue eyes S 1. His father has blue eyes, his mother had grey eyes and was dark

Mother dark brown hair (markedly darker than that of her husband) Over the left temple she has a small lock of white hair. Blue eyes S 7. Knows of no similar traits in her parents, but there is some albinism in the family, as to which you have doubtless got details from the father

Daughter no. 1 Nearly 12 years old, had a small white spot in the hair on the back of her neck at birth. This has long since disappeared. I did not see her or any other sib except the twin girls

Son no. 2 A boy aged 9, normal

Son no. 3 A boy aged 7, albino, not seen by me

Daughters, nos. 4 and 5 Twin girls, born 13-9-1926. Nellie is a pronounced albino, Jennie not. They have a striking family resemblance, but do not look entirely like identical twins. Nellie, the albino, is slightly taller and heavier, and the face is noticeably rounder and broader. She has excellent teeth while Jennie's teeth are very carious, the incisors, canines and two or three other being largely rotted away. Nellie has not albino eyes, but blue eyes S 8. Jennie's eyes are also blue S 7, corresponding closely to her mother's. Jennie has rather light brown hair, but it is said to have been definitely darker, when she was infant than it is now. Jennie has a marked double crown with dextral whorls. Nellie's pattern is a little confused and hard to make out, but she appears to have only one crown, and the direction of the whorl is not well defined. I really could not decide, whether it was dextral or sinistral. Nellie has pronounced albino hair, white with just a tinge of yellow towards the ends. There seemed to be little difference in texture between her hair and that of her sister. Nellie has a very pink complexion. Jennie's is more sallow like that of her mother. Nellie has very marked nystagmus and photophobia.

In einem folgenden Briefe schreibt Herr POPSON:

„Re the twins, Dr. WAITINGSFORD has replied merely saying:

„I remember this case very vividly and the twin girls were one divide ova and one placenta with an extreme excessive amount of fluid“

Der Vater schreibt mir folgendes: „Die 2 Albinos haben weisse Kopfhaare, Augenbrauen, Wimpern und Körperhaare. Die Augen

sind etwas rötlicher und sind immer in Bewegung, und in der Dämmerung sind sie leicht blau. Die Kinder sind lichtscheu (Fig. 35). Sie sehen sehr schlecht, müssen alles bis auf 6 cm. von den Augen halten.



FIG. 35

Die Haut ist sehr hell, kann die Sonne nicht ertragen. Meine Frau hat einige weisse Haare auf dem Kopf, weiter hat sie keine weisse Hautstellen."

Muttersvater erzählt mir, dass seine Tochter schon von Geburt an die weisse Haarlocke gehabt hat. Er weiss nicht mehr ob er oder seine verstorbene Frau auch etwas ähnliches oder einen weissen Hautfleck hatte. Seine andern Kinder haben nicht so etwas dergleichen.

130. Albinos 1 K und 1 M. Total 2 K und 1 M. G 1 und 2. B 4e Stufe. Verwandtschaft mit 37 (Fig. 14). Dieser Fall ist wichtig, weil der gemeinschaftliche Ahne in einer Seitenlinie wieder einen Albino hat.

131. Albinos 2 K und 2 M. Total 7 K und 8 M. G 1, 5, 6, und 14.

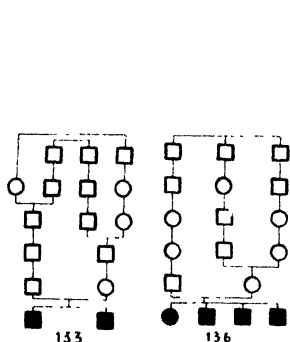


FIG. 36

FIG. 37

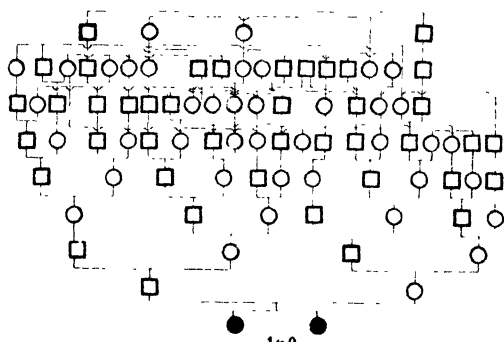


FIG. 38

132. Albinos 2 K. Total 7 K und 2 M. G 2 und 8.

133. Albinos 2 K. Total 7 K und 2 M. G 2 und 8. B 9e und 10e Stufe (Fig. 36).

134. Albinos 1 K und 1 M. Total 1 K und 2 M. G 1 und 2. Verwandtschaft mit 121 (Fig. 12). Ahnentafel mit Lucken.

135. Albino 1 M. Total 2 M. G 1. Ahnentafel mit Lucken.

136 Albinos 3 K und 1 M Total 5 K und 4 M G 2, 4, 5 und 8 B 10e Stufe zweimal (Fig. 37)

137 Albino 1 K Total 4 K und 3 M G 3 Ahnentafel mit Lücken

138 Albinos 2 M Total 2 M G 1 und 2.

139 Albino 1 M Total 1 M G 1 Ahnentafel mit Lücken

140 Albinos 2 M Total 2 M G 1 und 2 B verschiedene höhere Stufen (Fig. 38) Verwandtschaft mit 103 und 107 (Fig. 39) Beide Mädchen haben Nystagmus, Kopfwackeln, Fundi oculi pigmentlos, Kopfhaare, Augenbrauen und Wimpern pigmentlos

In diesen 140 Familien sind 702 Kinder geboren, 367 Knaben und 335 Mädchen, hiervon sind 216 Albinos, 115 Knaben und 101 Mädchen. Das Geschlechtsverhältnis ist also, dass von den Knaben 31,3% und von den Mädchen 30,2% Albinos sind. Ein Geschlechtsunterschied besteht also nicht.

Wir haben dann untersucht, ob es vielleicht einen Unterschied gibt in Geburtenfolgennummer. Zur Beantwortung dieser Frage haben wir folgende Tabelle zusammengestellt (siehe Tabelle Seite 114).

Zur Erklärung dieser Tabelle diene folgendes. Die letzten Buchstaben stellen die Anzahlen Albinos nach dem Geburtenfolgennummer da. Die Zahlen in Klammern stellen die Anzahlen da, wenn die Fälle gleichmässig über alle Folgenummer verteilt waren. Zum Beispiel waren in den Familien mit sechs Kindern 20 Albinos. Waren die Albinos gleichmässig verteilt, dann würde theoretisch die Zahl für jede Folgenummer $20 : 6 = 3\frac{1}{3}$ betragen. Also steht diese Zahl in Klammern. Wenn nun alle wirklichen Fälle und auch die theoretisch berechneten Fälle zusammengezählt werden, so bekommen wir Zahlen, die fast gleich gross sind (Reihen A und B). Wir können daher keinen Einfluss der Geburtenfolgennummer feststellen.

WAARDENBURG und viele anderen Autoren behaupten, dass der Albinismus totalis einfach rezessiv erblich ist. Um dies an unserem Material zu prüfen sind die Methoden nach WEINBERG und nach LENZ benutzt. Nach diesen Methoden ist das Verhältnis zwischen den Albinos und den Geschwistern

WEINBERG $1 : 3,8$. Also sind Albino $20,8\% \pm 3\sigma = 4,611$ Grenzen, 29,611 und 20,389.

LENZ $1 : 3,34$. Also sind Albino $23,1\% \pm 3\sigma = 4,764$ Grenzen, 29,764 und 20,236.

Die gefundenen Prozentzahlen fallen also innerhalb den Grenzen

Familien grösse	Geburtenfolgennummer der Albinos										To- tal
	1e K	2e K	3e K	4e K	5e K	6e K	7e K	8e K	9e K	10e K	
1	8 (8)										8
2	14 (13 ¹ / ₂)	13 (13 ¹ / ₂)									27
3	13 (9 ¹ / ₄)	11 (9 ¹ / ₄)	4 (9 ¹ / ₄)								28
4	9 (6 ³ / ₄)	6 (6 ³ / ₄)	8 (5 ³ / ₄)	4 (6 ¹ / ₄)							27
5	2 (5)	5 (5)	6 (5)	7 (5)	5 (5)						25
6	4 (3 ¹ / ₃)	1 (3 ¹ / ₃)	6 (3 ¹ / ₃)	4 (3 ¹ / ₃)	2 (3 ¹ / ₃)	3 (3 ¹ / ₃)					20
7	1 (2 ² / ₇)	3 (2 ² / ₇)	3 (2 ² / ₇)	3 (2 ² / ₇)	4 (2 ² / ₇)		2 (2 ² / ₇)				16
8	2 (2 ¹ / ₂)	4 (2 ¹ / ₂)	2 (2 ¹ / ₂)	2 (2 ¹ / ₂)	2 (2 ¹ / ₂)	3 (2 ¹ / ₂)	1 (2 ¹ / ₂)	4 (2 ¹ / ₂)			20
9		5 (1 ⁸ / ₉)	3 (1 ⁸ / ₉)	2 (1 ⁸ / ₉)	2 (1 ⁸ / ₉)			3 (1 ⁸ / ₉)	2 (1 ⁸ / ₉)		17
10		2 (1 ⁹ / ₁₀)			2 (1 ⁹ / ₁₀)	2 (1 ⁹ / ₁₀)	2 (1 ⁹ / ₁₀)	1 (1 ⁹ / ₁₀)	2 (1 ⁹ / ₁₀)	2 (1 ⁹ / ₁₀)	13
11			1 (1 ⁴ / ₁₁)	1 (1 ⁴ / ₁₁)			1 (1 ⁴ / ₁₁)	1 (1 ⁴ / ₁₁)			4
12				1 (1 ⁴ / ₁₁)	1 (1 ⁴ / ₁₁)				1 (1 ⁴ / ₁₁)	1 (1 ⁴ / ₁₁)	4
15	2 (2 ¹ / ₅)				2 (2 ¹ / ₅)	2 (2 ¹ / ₅)					5
to- tal	55 (54.96)	50 (46.96)	33 (33.4)	24 (24.13)	20 (17.36)	10 (12.52)	5 (6.5)	9 (6.72)	6 (4.2)	3 (2.37)	A
Theoretische Erwartung											B

des Zufalls, stimmen deshalb ganz gut überein mit der Annahme der einfachen Rezessivität.

Der stärkste Hinweis auf die Rezessivität ist wohl die hohe Konsanguinität der Eltern. Wie seltener die Eigenschaft vorkommt, um so wichtiger ist dieser Beweis. Nun ist Albinismus eine sehr seltene Abweichung. Wir wollen nicht behaupten, dass wir alle Fälle in Holland gesammelt haben, aber doch wohl sehr viele. Wir glauben wenig-

stens 50%, Dann wurden in unserem Lande etwa 400 Fälle vorkommen, das ist also 1 auf 20000 Personen

Nun ist in unserem Lande die Konsanguinität in vierter Stufe bekannt für die Jahre bis 1919. Nehmen wir nun die Jahre 1907-1918. In diesen 12 Jahren sind 531246 Ehen geschlossen, wovon 3491 konsanguin. Bei unseren 140 Familien mit Albinos besteht 15 mal Konsanguinität in der vierten Stufe. Wir können dann statistisch folgendes bemerken. Seien

	in den Jahren 1907-1918	mit Albinos
die Ehen	N = 531246	n = 140
davon konsanguin	3491	15
Die Frequenz der Konsanguinität ist dann	P = 0,006571	p = 0,1071

Von allen Ehen in 1917-1918 und den Ehen mit Albinos zusammen $P_z = 0,006598$

Die mittlere Abweichung ist dann

$$\sigma = \sqrt{0,006598 \cdot (1 - 0,006598)} \left| \frac{1}{531246} + \frac{1}{140} \right| = 0,006843$$

$$3\sigma = 3 \cdot 0,006843 = 0,020529$$

$$p - P = 0,100529 - 0,006598 = 0,093931$$

Weil der Unterschied zwischen P und p weit mehr als 3σ ist, ist Zufall ausgeschlossen, besteht also ein wirklicher Zusammenhang zwischen Konsanguinität und Albinismus.

Wenn der Albinismus rezessiv erblich ist, dann hat das Kind von beiden Eltern einen rezessiven Faktor geerbt. Selbstverständlich braucht der Albinismus nicht in den Aszendenz manifest gewesen zu sein, aber die Möglichkeit besteht. Es ist möglich, dass sowohl in der Familie des Vaters als in derjenigen der Mutter Albinismus vorkommt. Davon haben wir in unserem Material 7 Fälle gefunden, nämlich die Nummer 6, 52, 53, 70, 103, 107-139 und 140 (siehe die betreffenden Figuren).

Wir haben, wie schon oben gesagt, 15 Fälle, wo die Eltern Vetter und Consue sind und viele andere mit noch höheren Stufen von Blutverwandtschaft, wie aus folgender Tabelle hervorgeht:

Stufe	Nummer	Total
4e	11, 18, 20, 33, 40, 41, 42, 69, 73, 74, 77, 78, 79 (2 mal), 90, 130	15
5e	62	0
6e	2, 5, 9 (2 mal), 22, 35, 47, 70, 77 (2 mal), 99, 129	10
7e	80	1
8e	9, 14, 22, 28, 46 (3 mal), 60 (2 mal), 77, 82, 133	9
9e	133	1
10e	9, 13 (2 mal), 46 (5 mal), 77, 133, 136 (2 mal)	6
11e	41	1
12e	47	1
15e	41	1
16e	7	1

und dann gibt es noch einige (46, 77, 103, 107, 140), wo man wohl von Inzucht sprechen kann. Die betreffenden Figuren zeigen das deutlich. Es betrifft Albinos, die in kleinen Dörfern wohnen, wo die Sitte vorherrscht so viel wie möglich eine Frau aus dem Dorfe zu heiraten. Fig. 29 zum Beispiel betrifft 3 Fischerfamilien aus einem Fischerdorf am Nordsee, wo in den meisten Fällen ein junger Fischer eine Fischerstochter heiratet.

Die Verwandtschaft der Eltern besteht bei

Num.	46	6 mal
"	77	5 "
"	103	9 "
"	107	16 "
"	140	8 "

Weiter gibt es 17 Familien, worin 50% oder mehr der Geschwister Albinos sind und wohl

Num	Anzahl Geschwister	wovon Albinos
16	5	3
41	8	4
49	10	5
65	4	3
75	4	2
77	4	2
92	3	2
94	4	2
97	4	2
102	4	2
104	4	2
116	2	2
120	2	2
130	3	2
134	3	2
138	2	2
140	2	2

Wir nehmen hier nicht bei die Familien mit einem Kinde, dass Albinus ist. Dass wir diese 17 Fälle hier gesondert nennen, hat mehr einen historischen als wissenschaftlichen Grund. MAGNUS hat eine Albinofamilie mit 1 normalen Kinde und 7 Albinos beschrieben, FULTON eine mit 3 normalen und 5 Albinos, KATH WELBER eine mit 7 normalen und 6 Albinos (zitiert nach WAARDENBURG). PLATE hat sich über den Fall MAGNUS gewundert und findet es unbegreiflich. Wir stimmen WAARDENBURG bei, dass PLATE sich hier irrt. Wenn wir z.B. obengenannte 17 Familien zusammennehmen, dann finden wir bei total 68 Geschwistern 42 Albinos, also 62%. Man würde sich an diesem Prozentsatz eher für Dominanz entscheiden als für Rezessivität. Zwei Gründe sind dagegen. Erstens kann man die Wirkung des MENDELschen Gesetzes nur bei grossen Anzahlen feststellen. Bei unseren 140 nicht speziell ausgesuchten Familien sehen wir ein ganz anderes Verhältnis. Der zweite Grund ist dieser, dass eine Berechnung von 62% nicht richtig ist. WEINBERG war der Erste, der darauf hingewiesen hat. Wenn mehr als ein Fall

in einer Familie vorkommt, muss man eine andere Rechenmethode benutzen, und wohl eine der Methoden nach WEINBERG oder LENZ, wie wir es oben getan haben. Die meisten früheren Autoren haben dies nicht getan. Weder LAGLEYZE, noch DAVENPORT haben es so berechnet. SEYFFART hat das Material von 74 Stammbäumen nach WEINBERG's Methode berechnet. WAARDFENBURG schreibt, dass die Zahlenverhältnisse nach Korrektion der Auslesefehler sind, wie erwartet wurde. Er hat sie aber nicht publiziert; vielleicht, da SEYFFART es an einem grösseren Material schon getan hat.

Wir haben keinen einzigen Fall von direkter Erbllichkeit in unserem Material. Wohl haben wir bei Nummer 95 einen Albino genannt, der auch ein Albinokind haben soll zufolge Auskunft der Mutter. Wir haben diesen Fall aber nicht weiter untersucht, da die betreffende Familie in Indien wohnt.

Die Nummer 126 und 129 betreffen diskordante, zweieiige Zwillinge.

Die Nummer 95 und 129 sind aber auch aus andern Gründen sehr wichtig. Der Bruder des Albinos hat eine weisse Haarlocke, also Albinismus circumscriptus. Eine Schwester der Frau dieses Letzteren hat auch Albinismus circumscriptus. Da besteht kein Blutverwandtschaft zwischen diesen Eltern, so weit wir es zurückverfolgen konnten. Das Kind des Bruders mit Albinismus circumscriptus hat aber Totalalbinismus. Wir haben hier also in der einen Familie zwei Brüder, der eine mit Totalalbinismus, das rezessiv ist, der andere mit Albinismus circumscriptus, das dominant ist. Die Schwester der Frau dieses Letzteren hat auch Albinismus circumscriptus, während das Kind des Trägers der weissen Haarlocke und der phänotypisch normalen Frau das rezessive Totalalbinismus hat. Wir haben bei den Eltern der Träger der weissen Haarlocke keine weissen Hautstellen gefunden und auch bei Nachfragen eine negative Antwort bekommen. Dies ist nicht absolut beweisend, denn wir haben früher mehrere Male weisse Hautstellen gefunden bei Personen, ohne dass diese das gewusst haben.

Etwas ähnliches finden wir bei Fall 129, das auf unserer Bitte von Herin PAUL POPFNOE in Californien untersucht worden ist, wofür wir ihm hier gern unseren herzlichsten Dank aussprechen. Die Mutter der zwei Albinokinder hat eine weisse Haarlocke. Wir haben uns selbst davon überzeugen können, da sie uns das Haar zugeschickt

hat. Eine Tochter sollte nach der Mutter bei der Geburt auch eine weisse Haarlocke gehabt haben, die aber allmählich verschwunden ist. Dieses Verschwinden ist nicht selten; wir haben es bei andern Fällen auch gesehen. Muttersvater, ein Bauer, der jetzt schon grau ist, ist von uns untersucht worden; leider haben wir keine weisse Stelle finden können. Muttersmutter ist schon von mehr als 20 Jahren verstorben. Wir haben hier also auch eine Familie, worin Totalalbinismus und Albinismus *circumscriptus* neben einander vorkommen.

Nun ist die Frage, ob zwischen dem rezessiven Totalalbinismus und dem dominanten Albinismus *circumscriptus* genetisch Zusammenhang besteht. Unsere Fälle sind nicht die einzigen bekannten. Nach WAARDENBURG haben KOMAI und GATES bei direkten Verwandten von Albinos pigmentlose Hautstellen sowie eine weisse Haarsträhne gefunden. Ist dies alles nun Zufall? Sowohl Totalalbinismus als Albinismus *circumscriptus* sind selten. Bei unseren 140 Familien finden wir zwei, wo diese seltenen Anomalien zusammen vorkommen. Es ist sehr unwahrscheinlich, dass dieses Zufall ist. Wie muss es dann erklärt werden? Wahrscheinlich sind diese Personen mit Albinismus *circumscriptus* Heterozygoten, welche eine Ausnahme machen auf die meisten Heterozygoten, da diese Letzteren fast immer phaenotypisch normal sind. Bei diesen zwei Aa-Personen lässt sich a phaenotypisch gelten, sei es auch gering. Es ist wahrscheinlich dass diese 2 Personen ein Albinismus *circumscriptus* haben, das nicht dasselbe ist als das Albinismus *circumscriptus*, das dominant in Familien vorkommt, obwohl phaenotypisch Übereinstimmung besteht. Ob es hier ein anderes Gen betrifft, oder ob in oben beschriebenen 2 Fällen noch ein anderes Gen ausserdem eine Rolle spielt, ist nicht zu sagen.

Noch wollen wir folgendes bemerken.

Bei den Nummern 77 und 125 haben wir 2 Albinos gefunden, die Epilepsie haben.

Vielleicht sind im Material einzelne Fälle gonosomaler Augenalbinismus und vielleicht auch hereditärer, gonosomaler Nystagmus mit zufälligem Fundus flavus anwesend. Aber das schadet den gefundenen Geschlechtsverhältnissen fast nichts.

Es ist bisweilen schwer den Einfluss der Blutverwandtschaft zu beurteilen. So sind die Eltern des Falles 70 blutverwandt. Wahr-

scheinlich also haben diese Eltern von einem der gemeinschaftlichen Ahnen das betreffende Gen geerbt. Aber nun ist väterlicher- und mütterlicherseits auch ein Albino in der Familie. Die 2 Albinos des Falles 70 können also auch vom Vatersvater und Muttersvatersvater die Gene geerbt haben, und nicht von einem der gemeinschaftlichen Ahnen. Hier steht also die Bedeutung der Blutverwandtschaft in Frage. Dasselbe ist der Fall bei den Nummern 9 (Fig. 4 und Fig. 5), 40 (Fig. 51) und 46 (Fig. 17).

Wir wollen noch weisen auf Fall 130 (Fig. 14). Mit grosster Wahrscheinlichkeit kann gesagt werden, dass die Eltern vom gemeinschaftlichen Grossvater das Gen geerbt haben, weil dieser in einer Seitenlinie noch mit einer Albino verwandt ist.

Bei den Fällen 12, 66, 70, 92, 99, 117, 119 und 120 ist kein Nystagmus gefunden. Diese Abwesenheit gehört nicht zum klinischen Bild, und es ist die Frage, ob hier wohl ein *n* vorliegt oder ein unregelmässig-dominanter *Dr*.

Wir wollen nicht behaupten, dass mit dieser Studie die Frage der Erbllichkeit des Albinismus ganz aufgeklärt ist. Im Gegenteil, wir haben viele Schwierigkeiten begegnet, welche wir nicht zu erklären wussten, weil viele Data nicht bekannt waren. Eine noch genauere, detaillierte Untersuchung kann vielleicht diese schwierigen Fragen lösen.

A CASE OF BUDVARIATION IN PHASEOLUS CAUSED BY A TRANSITORY PLASMATIC CHANGE

by

M. J. SIRKS

(Groningen, Netherlands)

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In a vegetable-nursery among a group of *Phaseolus vulgaris* (Var. „Slagzwaard”) in 1925 a plant has been found which showed a remarkable budvariation. This budvariation seemed important for three reasons: 1) most budvariations are known in complicated heterozygous individuals, by what the study of the offspring of this budvariation as compared with that of the other parts of the same plant is extremely impeded, while it may be assumed that the bean-plant is of a homozygous nature in the highest degree, 2) the budvariation bore two well developed fruits and 3) the budvariation affected measurable quantitative characters, a case which is scarcely mentioned in literature.

The plant (fig. 1) had developed three fruits at the basal part of the stem, two of which (I and II) differed conspicuously from the original type (III). The seeds formed in these aberrant pods I and II (each 6) were sowed separately (2622.1-6 and 2623.1-6), as also three seeds from the normal pod (2624.1-3). Measurements of the pods, formed by these fifteen plants, are given in tables 1 and 2 and showed immediately a tendency towards inheritance. The 89 pods of the six plants in group I (fig. 2) supplied the following numbers: length $M = 13.83 \text{ cm.} \pm 1.27$, width $M = 1.550 \pm 0.111$, 91 pods of group II (fig. 3) had for the length $M = 15.43 \pm 1.07$ and for the width $M = 1.648 \pm 0.126$, while the 45 pods of the normal group III (fig. 4) produced $M = 18.72 \pm 1.39$ and $M = 2.171 \pm 0.134$. The

relations between I and II were: $D : m_{Diff} \approx 9.1$ resp. 5.5, for II and III $D : m_{Diff} \approx 13.9$ resp. 21.9. The distribution of the pods of these 15 plants is pictured graphically in fig. 5 (classes 10.0–10.5 till 22.0–22.5, resp. 1.2 till 2.6).

In the second generation, raised by selfing, D_2 , 6 + 6 + 3 families have been grown, comprising 315 + 341 + 186 individuals (group I,

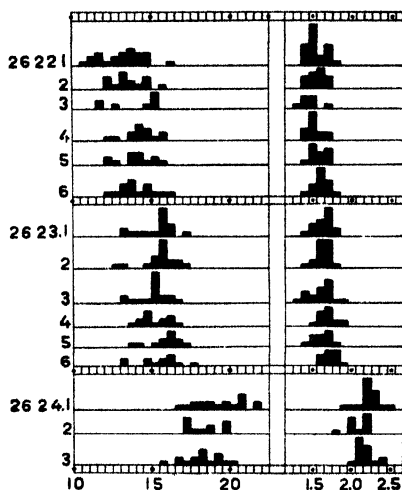


FIG. 5 Distribution of the 6 + 6 + 3 D_1 -plants according to their podlengths resp. podwidths

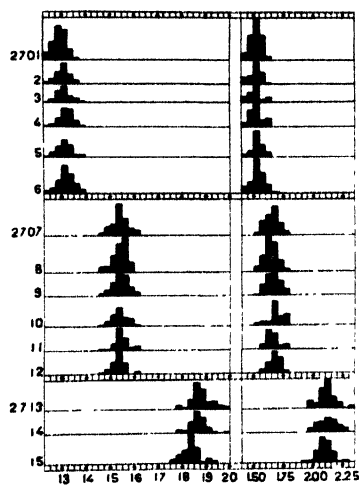


FIG. 6 Distribution of the mean values of 6 + 6 + 3 D_2 -families according to their podlengths resp. podwidths

2701–2706; group II, 2707–2712; group III, 2713–2715). In this generation the differences in dimensions of pods remained as they were in the D_1 ; the mean values in group I were decreased even more than such was the case in the preceding generation. The distribution of individual means in these 15 D_2 -families according to the classes 12.26–12.50 till 19.75–20.00, resp. 1.40–1.45 till 2.30–2.35 is shown in figure 6. These preliminary results seemed to prove a full inheritance of the aberrant dimensions of pods.

From the third generation on (by selfing, D_3 , grown in 1928) however a change could be observed. The mean values of the families in group I had increased a little, but they did not stand out above the individual means in the first selfed generation (D_1); the mean-values



Fig. 1. The original plant showing the budvariation



B.2622-1



B.2622-2



B.2622-3

Fig. 2. Pods of three D_1 -plants of group 1.

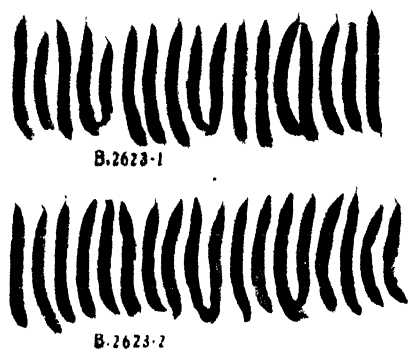


Fig. 3. Pods of two D_1 -plants of group II



Fig. 4. Pods of two D_1 -plants of group III.

of the families in group II however were considerably higher than the numbers of the preceding generations, while they came nearer to the values of the original type. The dimensions in group III had not changed at all. In the following generations, all of them obtained by selfing, this phenomenon has been observed to a high degree, as

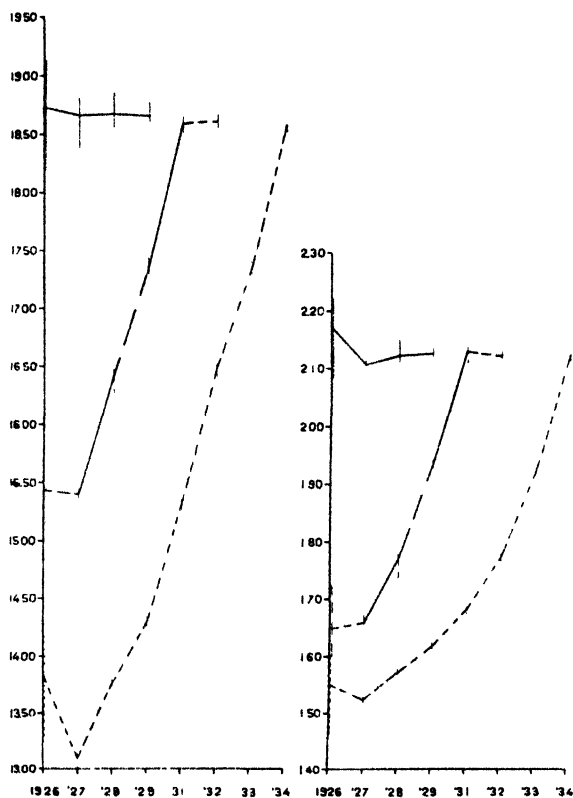


FIG. 7. The mean values for podlengths, resp. podwidths in the succeeding generations D_1 – D_8 (Dotted line group I, broken line group II, solid line group III).

is shown in tables 3–8; beginning with the generation D_4 the mean values of the families in groups I and II got more and more near to the mean values of group III (the original type). In group I the normal dimensions were attained in the eighth generation after selfing (D_8), in group II this was the case already in D_5 . Figure 7 represents the graphs, showing the mean values of families in successive generations,

both for the length and width of pods, while the main numbers from tables 1-8 have been summarized in table A.

TABLE A. MEANVALUES OF THE OFFSPRING, OBTAINED BY SELFING

	n	Podlength		Podwidth	
		M	$\pm \sigma$	M	$\pm \sigma$
<i>Group I</i>					
D ₁	6	13.83	± 1.27	1.550	± 0.111
D ₂	315	13.093	± 0.317	1.5235	± 0.0521
D ₃	501	13.758	± 0.307	1.5732	± 0.0532
D ₄	536	14.290	± 0.329	1.6168	± 0.0557
D ₅	573	15.322	± 0.345	1.6831	± 0.0616
D ₆	562	16.498	± 0.297	1.7785	± 0.0546
D ₇	580	17.377	± 0.297	1.9247	± 0.0605
D ₈	558	18.600	± 0.321	2.1291	± 0.0599
<i>Group II</i>					
D ₁	6	15.43	± 1.07	1.648	± 0.126
D ₂	341	15.406	± 0.283	1.6596	± 0.0585
D ₃	471	16.428	± 0.335	1.7716	± 0.0582
D ₄	482	17.368	± 0.349	1.9323	± 0.0531
D ₅	489	18.603	± 0.354	2.1334	± 0.0728
D ₆	516	18.616	± 0.297	2.1248	± 0.0682
<i>Group III</i>					
D ₁	3	18.72	± 1.39	2.171	± 0.134
D ₂	186	18.662	± 0.374	2.1081	± 0.0746
D ₃	538	18.686	± 0.376	2.1221	± 0.0683
D ₄	522	18.674	± 0.345	2.1270	± 0.0663

Since the results of 1926 seemed to show that the aberrant form of pods was not due to a modification, but all appearances pointed to a real inheritance of this change in character, it was decided to make crossings between the three types in both reciprocal ways. The results of the F₁-plants grown in 1928, at first were a little confusing: the dimensions of pods showed generally a strong maternal inheritance, but in those crosses in which plants of groups I or II had been used as motherplants, the meanvalues of the F₁-generations were found

to be much higher than those of the mothers (for group I 13.629 and 13.670 to 12.29 and for group II 16.250 and 16.375 to 14.83). More especially the behaviour of the cross II \times I was not very clear, as the F_1 excelled both parents.

In the four crosses in which abnormal mothers had been used for the crosses, the meanvalues obtained in the F_1 -generations, came much nearer to the normal type which phenomenon was increased in the next generations. The results obtained in this set of crossings are given in full in tables 9-14, where the meanvalues of the families in the offspring of these crosses have been tabulated in table B.

TABLE B MEANVALUES IN THE OFFSPRING FROM CROSSES

	n	Podlengths M : σ		Podwidths M : σ	
<i>Group I \times Group III</i>		(12.29	18.15)	(1.542	\times 2.241)
F_1	7	13.629	\pm 0.303	1.5866	\pm 0.0720
F_2	277	14.339	\pm 0.309	1.6261	\pm 0.0624
F_3	269	15.412	\pm 0.280	1.6729	\pm 0.0617
F_4	282	16.361	\pm 0.317	1.7716	\pm 0.0634
F_5	270	17.390	\pm 0.317	1.9293	\pm 0.0606
<i>Group III \times Group I</i>		(18.15	\times 12.29)	(2.241	\times 1.542)
F_1	5	18.678	\pm 0.324	2.1414	\pm 0.0535
F_2	273	18.629	\pm 0.302	2.1228	\pm 0.0603
<i>Group I \times Group II</i>		(12.29	\times 14.83)	(1.542	\times 1.595)
F_1	11	13.670	\pm 0.257	1.5705	\pm 0.0498
F_2	282	14.365	\pm 0.306	1.6335	\pm 0.0607
F_3	269	15.412	\pm 0.307	1.6839	\pm 0.0586
F_4	273	16.389	\pm 0.320	1.7811	\pm 0.0618
F_5	271	17.387	\pm 0.299	1.9292	\pm 0.0618
<i>Group II \times Group I</i>		(14.83	\times 12.29)	(1.595	\times 1.542)
F_1	10	16.250	\pm 0.321	1.7900	\pm 0.0635
F_2	271	17.347	\pm 0.293	1.9320	\pm 0.0630
F_3	260	18.652	\pm 0.326	2.1393	\pm 0.0611
F_4	293	18.690	\pm 0.337	2.1377	\pm 0.0624
<i>Group II \times Group III</i>		(14.83	\times 18.15)	(1.595	\times 2.241)
F_1	8	16.375	\pm 0.250	1.7937	\pm 0.0496

	n	Podlengths M \pm σ	Podwidths M \pm σ
F ₂	269	17.459 \pm 0.305	1.9453 \pm 0.0660
F ₃	272	18.733 \pm 0.327	2.1342 \pm 0.0618
F ₄	266	18.744 \pm 0.346	2.1303 \pm 0.0615
Group III \times Group II		(18.15 \times 14.83)	(2.241 \times 1.595)
F ₁	9	18.680 \pm 0.258	2.1472 \pm 0.0583
F ₂	274	18.758 \pm 0.321	2.1308 \pm 0.0558

As a whole the outcome of these crosses has been entirely in keeping with those of the selfed generations: in all the crosses, in which the normal type (III) had been used as a mother, the entire offspring belonged to the same type, while those descending from abnormal mothers, shifted continuously in the same direction towards the normal values.

It seems to me that the only possible conclusion which may be drawn from the above-mentioned facts, points to a transitory change of the plasmatic nature in a basal part of the original individual, which change is not a common modification, neither a plasmatic mutation, because it is inherited in some degree for a few generations, but it disappears in succeeding generations, both after selfing and after crossing, by which the normal dimensions of the pods are re-established.

Of course it is impossible to judge of the essential nature of this phenomenon; it may be classified as a case of „Dauermodifikation“ (permanent modifications) or it may be considered a result of plasmatic exhaustion, but such a determination could not imply any further knowledge of the causal processes. Perhaps the best paraphrase would be considering the change as a transitory weakening of the reactionpower of the plasm in face of the genotypical factors for dimensions of pods. In my studies in *Vicia Faba* (1931, p. 372) I found that the subspecies *V. F. major* and *V. F. minor* do show analogous differences in reactionpower, towards genes for growth, which differences were fully inherited, but the transitory character in the budvariation studied in this paper, does not show any trace of real inheritance.

In *Phaseolus* a few cases of budvariation only have been mentioned in literature, most of which were relating to characters of chlorophyll, which were caused by a change in the plastids and therefore showed a somewhat irregular behaviour. As far as I know the only other case is that of the „*angustifolia*”-budvariation of JOHANNSEN (1909) which could be observed already in the first leaf following the cotyledons and which developed into a well-formed sprout bearing *angustifolia*-leaves. However this sprout did not bear any fruit, while among the offspring from the normal parts of the same individual no abnormal plants could be observed, so that the real nature of this budvariation is entirely unknown.

The phenomenon discussed above seems to approach more the results of HOFMANN (1927) which have been obtained by a treatment of seeds and young plants of the Navy bean with a 0.75 percent chloral solution. It is true that HOFMANN did not find any case of budvariation, while the entire individuals were changed regarding their habitus, form of leaves &c. These abnormalities were inherited during a few generations only; after the sixth generation the offspring from selfed plants had reached again quite normal characters. Crosses of normal plants as mothers with pollen of chloral-treated fathers produced normal plants only; the reciprocal crosses have not been mentioned in HOFMANN's paper. The results of HOFMANN seem to be partly analogous to those above-mentioned; both are examples of a transitory change in the nature of the plasm, but in HOFMANN's studies the cause of the change was well-known and the changes seized the plant as a whole, while in my materials the cause is unknown and the phenomenon was limited to a part of the plant only.

LITERATURE CITED

- HOFMANN, F. W., 1927. Some attempts to modify the germ plasm of *Phaseolus vulgaris* (Genetics, XII, 1927, p. 284-295)
JOHANNSEN, W., 1909. Über Knospenmutation bei *Phaseolus* (Zschr. ind. Abst. u. Vererb. Lehre, I, 1909, p. 1-10)
SIRKS, M. J., 1931. Beiträge zu einer genotypischen Analyse der Ackerbohne, *Vicia Faba* L. (Genetica, XIII, 1931, p. 209-631)

TABLE 1. LENGTH OF PODS D_1

plant	number of pods	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
2622 1	23	1	2	3	1	2	3	4	3	3	-		1			
2622 2	15				3	1	4	2	1	3	-	1				
2622 3	8			2	-	1	-	-		1		4				
I 2622 4	14				1	1	-	2	4	3	1	2				
2622 5	13				2	1		3	3	1	2	1				
2622 6	16				1	1	3	4	1	3	1	1	1			
Total	89	1	2	5	8	7	10	15	12	14	4	9	2			
2623 1	17						2	1	1	1	1	7	3		1	
2623 2	18					1	1	-	-	1	3	7	2	2	1	
2623 3	18						2	1	1	1	8	2	2	1		
II 2623 4	14							1	2	4	1	2	3	1		
2623 5	12							1	1		1	2	4	2	1	
2623 6	12						2	-		2	1	2	3	1		1
Total	91					1	7	4	5	9	15	22	17	7	3	1
2624 1	18													1	1	2
III 2624 2	11														4	1
2624 3	16											1	-	2	1	2
Total	45											1	-	3	6	5

ss 1 = 10.6-11.0; class 23 = 21.6-22.0)

18	19	20	21	22	23	M	σ	m
						13.23	1.36	
						13.62	1.03	
						14.25	1.73	
						14.32	0.96	
						14.02	1.07	
						14.09	1.05	
						13.83	1.27	0.1357
						15.34	1.09	
						15.56	1.08	
						15.11	0.92	
						15.25	0.89	
						15.62	0.92	
						15.25	1.28	
						15.43	1.07	0.1122
1	2	1	4		2	19.11	1.49	
	3					18.34	1.02	
3	1	1				18.19	1.07	
4	6	2	4		2	18.72	1.39	0.2075
								Diff = 1.60 m_{Diff} 0.1761
								D m_{Diff} = 9.1
								Diff = 3.29 m_{Diff} 0.2359
								D m_{Diff} = 13.9

For table 2 see page 140

TABLE 3. INDIVIDUAL MEANS OF PODLENGTHS IN GROUP I

[illegible]

TABLE 3 (Continued).

[illegible]

13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	$\pm \sigma$
		3	5	19	14	11	5										16 550 \pm 0 318
		1	6	21	15	7	2										16 505 \pm 0 264
		3	9	17	19	5	1										16 454 \pm 0 271
		5	7	22	16	6	2										16 448 \pm 0 295
		2	8	20	13	9	4										16 513 \pm 0 306
		4	9	19	16	7	3										16 470 \pm 0 307
			9	21	17	8	4										16 527 \pm 0 275
		3	11	16	14	9	5										16 504 \pm 0 332
		2	9	20	15	7	3										16 478 \pm 0 291
		1	6	22	13	8	4										16 528 \pm 0 286
		24	79	197	152	77	33										16 498 \pm 0 297
						1	5	14	20	11	6	2					17 383 \pm 0 319
							2	16	23	9	5	1					17 384 \pm 0 263
						1	3	13	21	12	7						17 392 \pm 0 284
							8	12	19	15	5						17 363 \pm 0 289
							5	11	22	13	6	1					17 405 \pm 0 286
						3	7	10	21	14	3						17 319 \pm 0 308
							6	9	23	10	7						17 389 \pm 0 284
							9	11	18	13	6	2					17 383 \pm 0 329
							3	15	21	12	8	1					17 416 \pm 0 285
						2	6	13	22	1	5						17 333 \pm 0 299
						7	54	124	120	210	58	7					17 377 \pm 0 297
										1	3	7	8	19	13	5	18 572 \pm 0 349
											4	5	9	18	11	7	18 607 \pm 0 350
											3	6	7	21	12	4	18 588 \pm 0 316
											1	5	12	18	14	3	18 601 \pm 0 280
											2	4	11	22	10	7	18 621 \pm 0 304
											3	7	10	18	11	5	18 570 \pm 0 329
											4	6	9	21	12	5	18 596 \pm 0 338
											3	7	12	17	15	3	18 564 \pm 0 315
											1	7	8	20	14	8	18 647 \pm 0 312
											2	6	10	19	13	7	18 621 \pm 0 319
										1	26	60	96	193	125	54	18 600 \pm 0 321

TABLE 4. INDIVIDUAL MEANS OF PODWIDTHS IN GROUP I (D₂-

Parent	Mean of parent	No.	number of plants	Class		3	4	5	6
				1	2				
2622 1	1.54	2701	85	6	21	34	21	3	
2622 2	1.55	2702	43	6	7	19	10	1	
2622.3	1.46	2703	33	5	8	14	2	4	
2622 4	1.52	2704	45	3	13	18	5	6	
2622 5	1.57	2705	42	2	5	21	10	4	
2622 6	1.60	2706	67	5	9	28	17	7	1
			315	27	63	134	65	25	1
2701 1	1.542	2801	59		4	16	23	11	5
2701 2	1.483	2802	46		6	12	16	9	3
2701 3	1.617	2803	52		3	13	21	12	3
2701 4	1.431	2804	43		2	9	17	10	4
2701 5	1.524	2805	48		3	10	19	14	2
2704 1	1.592	2806	41		2	9	15	11	4
2704 2	1.539	2807	53		5	12	20	10	4
2704.3	1.481	2808	58	1	6	13	22	11	5
2704 4	1.423	2809	52		5	17	18	9	3
2704 5	1.626	2810	49		3	14	19	10	3
			501	1	39	125	190	107	36
2802 1	1.651	2901	53		2	7	16	18	6
2802 2	1.683	2902	45			4	10	17	9
2802 3	1.587	2903	58		1	5	17	22	10
2802 4	1.632	2904	55			8	14	20	7
2802 5	1.498	2905	54		2	5	12	19	11
2806 1	1.619	2906	53			6	11	21	12
2806.2	1.523	2907	58			5	15	23	13
2806 3	1.475	2908	50			4	16	17	11
2806.4	1.666	2909	55		2	4	11	20	12
2806.5	1.554	2910	55		1	6	13	22	10
			536		8	54	135	199	101
2903.1	1.476	3101	55				8	13	22
2903 2	1.618	3102	53				4	10	19
2903.3	1.537	3103	57				3	9	24
2903 4	1.726	3104	60				5	14	21
2903 5	1.641	3105	55				6	11	18
2909 1	1.724	3106	59			2	5	10	19
2909.2	1.625	3107	58				5	12	23
2909.3	1.487	3108	59				6	11	22
2909 4	1.691	3109	58				2	12	24
2909.5	1.587	3110	59				4	13	21
			573			2	48	115	213

927-1934) (Class 1 = 1.40-1.34; class 18 = 2.25-2.30)

8	9	10	11	12	13	14	15	16	17	18	M \pm σ
											1 5203 \pm 0 0470
											1 5169 \pm 0 0497
											1 5129 \pm 0 0577
											1 5228 \pm 0 0547
											1 5357 \pm 0 0469
											1 5362 \pm 0 0545
											1 5235 \pm 0 0521
											1 5725 \pm 0 0516
											1 5652 \pm 0 0548
											1 5740 \pm 0 0485
											1 5843 \pm 0 0542
											1 5771 \pm 0 0478
											1 5823 \pm 0 0512
											1 5760 \pm 0 0599
											1 5690 \pm 0 0573
											1 5635 \pm 0 0516
											1 5709 \pm 0 0493
											1 5732 \pm 0 0532
											1 6042 \pm 0 0594
											1 6261 \pm 0 0553
											1 6129 \pm 0 0527
											1 6131 \pm 0 0597
											1 6185 \pm 0 0609
											1 6203 \pm 0 0548
											1 6181 \pm 0 0486
											1 6160 \pm 0 0497
											1 6241 \pm 0 0606
											1 6141 \pm 0 0545
											1 6168 \pm 0 0557
4	1										1 6650 \pm 0 0583
6	2										1 6863 \pm 0 0604
7	3										1 6916 \pm 0 0595
5	2										1 6791 \pm 0 0593
4	3										1 6813 \pm 0 0603
3	5										1 6835 \pm 0 0708
7	4										1 6845 \pm 0 0660
7	1										1 6801 \pm 0 0629
5	4										1 6897 \pm 0 0594
3	4										1 6843 \pm 0 0613
51	29										1 6831 \pm 0 0616

Table 4 (Continued)

Parent	Mean of parent	No	number of plants	Class					
				1	2	3	4	5	6
3101.1	1 596	3201	57					1	6
3101 2	1 743	3202	52						3
3101 3	1 634	3203	54					1	5
3101 4	1 592	3204	58						7
3101.5	1 814	3205	56						3
3107 1	1.745	3206	58						2
3107 2	1 624	3207	59						4
3107 3	1 691	3208	58						8
3107 4	1 830	3209	56						7
3107.5	1 683	3210	54						5
			562					2	50
3203 1	1 873	3301	59						
3203.2	1 685	3302	56						
3203 3	1 821	3303	57						
3203 4	1 743	3304	59						
3203 5	1 897	3305	58						
3210 1	1 855	3306	58						
3210 2	1.841	3307	55						
3210.3	1 698	3308	59						
3210 4	1.882	3309	60						
3210 5	1 777	3310	59						
			580						
3302 1	1.923	3401	56						
3302 2	1 876	3402	55						
3302 3	1 965	3403	53						
3302 4	2.034	3404	53						
3302 5	1 834	3405	56						
3308 1	2 072	3406	54						
3308 2	1.845	3407	59						
3308 3	1.937	3408	57						
3308.4	2 046	3409	58						
3308.5	1 898	3410	57						
			558						

	9	10	11	12	13	14	15	16	17	18	M \pm σ
1	13	3									1.7697 \pm 0.0543
3	15	4									1.7798 \pm 0.0510
2	10	5									1.7685 \pm 0.0579
	17	4									1.7764 \pm 0.0549
3	12	3									1.7723 \pm 0.0476
5	11	4									1.7742 \pm 0.0471
7	20	5									1.7826 \pm 0.0526
	11	9									1.7784 \pm 0.0615
1	13	6									1.7759 \pm 0.0578
2	9	5	1								1.7750 \pm 0.0569
1	131	48	1								1.7785 \pm 0.0546
<hr/>											
	7	11	20	13	6	1					1.9250 \pm 0.0631
	5	10	22	15	4						1.9277 \pm 0.0521
	5	13	19	16	3						1.9215 \pm 0.0553
2	7	14	21	10	5						1.9131 \pm 0.0606
	5	16	17	14	5	1					1.9259 \pm 0.0583
3	4	13	20	11	6	1					1.9241 \pm 0.0656
	3	11	21	9	7	4					1.9413 \pm 0.0654
	6	10	24	12	5	2					1.9301 \pm 0.0595
2	4	11	22	15	5	1					1.9275 \pm 0.0608
	8	9	25	10	6						1.9199 \pm 0.0591
1	54	118	211	125	52	10					1.9247 \pm 0.0606
<hr/>											
					3	11	22	12	8		2.1348 \pm 0.0538
					6	12	17	15	5		2.1259 \pm 0.0568
				1	5	12	20	9	6		2.1212 \pm 0.0589
				1	4	8	21	11	5	3	2.1354 \pm 0.0647
					5	11	23	10	5	2	2.1205 \pm 0.0593
				4	3	10	18	14	4	1	2.1222 \pm 0.0609
					4	13	24	11	6	1	2.1292 \pm 0.0555
				1	5	9	21	13	5	3	2.1338 \pm 0.0649
					5	11	19	16	6	1	2.1336 \pm 0.0581
					6	12	22	10	7		2.1250 \pm 0.0570
				7	46	109	207	121	57	11	2.1291 \pm 0.0599

TABLE 5. INDIVIDUAL MEANS OF PODLENGTHS IN GROUP II (I.

Parent	Mean of parent	No	number of plants	Class		12	13	14	15	16	17
				10	11						
2623 1	15.34	2707	68	2	7	9	26	14	6	4	
2623 2	15.56	2708	81	4	4	13	23	29	8		
2623 3	15.11	2709	64	2	5	11	19	17	7	3	
2623 4	15.25	2710	43	1	4	9	15	7	6	1	
2623 5	15.62	2711	40	1	1	5	18	10	2	3	
2623 6	15.25	2712	45	1	4	10	19	9	—	2	
			341	11	25	57	120	86	29	13	
2708 1	14.57	2811	47					5	4	11	16
2708.2	14.83	2812	33						5	7	13
2708 3	15.78	2813	37						2	8	15
2708 4	15.38	2814	40						4	5	14
2708.5	15.03	2815	56					2	3	10	17
2711 1	15.19	2816	57					4	4	13	22
2711.2	14.86	2817	42						2	8	18
2711.3	16.13	2818	52						3	8	19
2711.4	14.68	2819	55					4	2	7	15
2711 5	15.41	2820	52						1	6	14
			471					15	30	84	163
2813 1	16.43	2911	47								
2813 2	16.03	2912	50								
2813 3	16.79	2913	53								
2813 4	15.84	2914	52								
2813 5	16.29	2915	56								
2817.1	16.39	2916	45								
2817.2	16.62	2917	47								
2817 3	16.21	2918	41								
2817.4	16.09	2919	45								
2817.5	16.87	2920	46								
			482								
2912 1	17.41	3111	52								
2912 2	16.93	3112	46								
2912 3	16.57	3113	41								
2912 4	17.19	3114	39								
2912.5	18.13	3115	56								
2920.1	16.74	3116	54								
2920.2	17.73	3117	57								
2920.3	17.09	3118	58								
2920.4	18.09	3119	45								
2920.5	17.46	3120	41								
			489								

7-1932) (Class 10 -- 14.51-14.75; class 30 -- 19.51-19.75)

20	21	22	23	24	25	26	27	28	29	30	M + σ
											15 408 \pm 0 343
											15 412 \pm 0 310
											15 425 \pm 0 312
											15 387 \pm 0 328
											15 456 \pm 0 303
											15 341 \pm 0 292
											15 406 \pm 0 283
1											16 279 \pm 0 352
											16 337 \pm 0 296
											16 395 \pm p 249
1											16 450 \pm 0 307
3											16 446 \pm 0 346
2											16 322 \pm 0 334
1											16 417 \pm 0 277
4											16 485 \pm 0 319
5											16 465 \pm 0 383
3	2										16 587 \pm 0 319
20	2										16 428 \pm 0 335
15	13	7	3								17 263 \pm 0 295
11	16	5	5	4							17 350 \pm 0 391
9	21	8	5	3							17 331 \pm 0 329
10	17	12	7	2							17 442 \pm 0 310
7	16	13	8	1	3						17 460 \pm 0 401
7	13	6	4	3							17 314 \pm 0 402
10	14	9	2	1							17 269 \pm 0 349
8	15	6	3	3							17 375 \pm 0 354
9	17	8	6	1							17 408 \pm 0 301
11	19	6	4	2							17 375 \pm 0 290
97	161	80	47	20	3						17 368 \pm 0 349
		1	3	3	10	17	9	6	3		18 630 \pm 0 387
			2	5	9	16	10	3	—	1	18 598 \pm 0 339
			3	1	10	15	9	1	2		18 601 \pm 0 330
			1	1	7	17	10	2	—	1	18 587 \pm 0 292
			3	5	9	18	8	7	4	2	18 687 \pm 0 419
		1	4	6	11	17	8	4	3		18 560 \pm 0 394
		1	1	7	10	20	11	4	1	2	18 621 \pm 0 379
			4	7	13	21	8	3	2		18 539 \pm 0 341
			2	4	10	19	7	3			18 564 \pm 0 289
				4	7	18	9	3			18 625 \pm p 259
		3	23	43	96	178	89	36	15	6	18 603 \pm 0 354

Table 5 (Continued).

Parent	Mean of parent	No.	number of plants	Class		12	13	14	15	16	17
				10	11						
3114 1	18 29	3211	53								
3114 2	19 19	3212	58								
3114 3	17 82	3213	47								
3114 4	18 93	3214	51								
3114 5	18 07	3215	50								
3117 1	19 31	3216	54								
3117.2	18 14	3217	57								
3117 3	17 93	3218	43								
3117 4	18.31	3219	52								
3117 5	18 69	3220	57								
			516								

TABLE 2. WIDTH OF PODS D₁

plant	number of pods	Class									
		1	2	3	4	5	6	7	8	9	10
2622 1	23		5	10	2	5	1				
2622 2	15		3	4	5	3					
2622 3	8	1	3	3		1					
2622 4	14		3	7	2	2					
2622 5	13		1	5	3	4					
2622 6	16		1	3	7	4	1				
Total	89	1	16	32	19	19	2				
2623 1	17		1	3	4	7	2				
2623.2	18		1	2	7	7	1				
2623 3	18	1	3	2	4	6	1	1			
2623 4	14			1	4	5	2	2			
2623.5	12		1	3	3	4	1				
2623.6	12				3	4	4	1			
Total	91	1	6	11	25	33	11	4			
2624.1	18							1	1	1	
2624.2	11						1		4	1	
2624.3	16								1	7	
Total	45						1	1	6	9	

	20	21	22	23	24	25	26	27	28	29	30	$M \pm \sigma$
				2	4	11	19	13	1	3		$18\,620 \pm 0\,324$
				1	3	14	21	12	6	1		$18\,642 \pm 0\,293$
				2	6	9	17	11	2			$18\,561 \pm 0\,298$
				3	3	7	18	15	3	2		$18\,649 \pm 0\,333$
				1	4	9	20	11	1	3	1	$18\,655 \pm 0\,338$
				2	5	12	22	10	2		1	$18\,579 \pm 0\,308$
				4	4	8	21	13	7			$18\,621 \pm 0\,330$
				2	3	10	16	9	1	2		$18\,596 \pm 0\,320$
				1	4	9	22	14	2			$18\,616 \pm 0\,259$
			1	1	5	8	20	11	5			$18\,606 \pm 0\,320$
			1	19	41	97	196	119	30	11	2	$18\,616 \pm 0\,297$

ass 1 = 1.3; class 13 2.5)

12	13	$M \pm \sigma$	m
		$1\,543 \pm 0\,107$	
		$1\,553 \pm 0\,103$	
		$1\,462 \pm 0\,111$	
		$1\,521 \pm 0\,094$	
		$1\,577 \pm 0\,097$	
		$1\,606 \pm 0\,094$	
		$1\,550 \pm 0\,111$	0 0119
		$1\,635 \pm 0\,108$	
		$1\,628 \pm 0\,096$	
		$1\,600 \pm 0\,153$	
		$1\,700 \pm 0\,113$	
		$1\,608 \pm 0\,111$	
		$1\,725 \pm 0\,092$	
		$1\,648 \pm 0\,126$	0 0132
1	1	$2\,222 \pm 0\,131$	
		$2\,081 \pm 0\,148$	
2		$2\,175 \pm 0\,109$	
3	1	$2\,171 \pm 0\,134$	0 0200

$D_{\text{diff}} = 0\,098$ $m_{D_{\text{diff}}} =$
 $\approx 0\,0178$
 $D : m_{D_{\text{diff}}} = 5\,5$

$D_{\text{diff}} = 0\,523$ $m_{D_{\text{diff}}} =$
 $\approx 0\,0239$
 $D : m_{D_{\text{diff}}} = 21.9$

TABLE 6. INDIVIDUAL MEANS OF PODWIDTHS IN GROUP II (D₂-D₆, 1927-19
(Class 3 = 1.51-1.55; class 19 = 2.31-2.35)

Parent	Mean of parent	No.	number of plants	Class																M ±	
				3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18		19
2623.1	1.635	2707	68	2	12	17	24	10	3												1.6522
2623.2	1.628	2708	81	4	13	29	24	9	2												1.6417
2623.3	1.600	2709	64	1	9	18	19	12	5												1.6617
2623.4	1.700	2710	43	2	3	3	20	7	8												1.6843
2623.5	1.608	2711	40		4	16	13	3	4												1.6587
2623.6	1.725	2712	45		3	9	18	13	2												1.6772
			341	9	44	92	118	54	24												1.6596
2708.1	1.632	2811	47				6	11	18	9	3										1.7672
2708.2	1.595	2812	33				3	5	13	7	5										1.7847
2708.3	1.547	2813	37				1	4	17	9	6										1.7953
2708.4	1.762	2814	40					5	18	10	7										1.7994
2708.5	1.714	2815	56					4	6	23	15	8									1.7906
2711.1	1.593	2816	57			6	9	19	16	5	2										1.7341
2711.2	1.642	2817	42					5	14	15	6	2									1.7584
2711.3	1.781	2818	52			2	4	17	18	7	4										1.7603
2711.4	1.732	2819	55			2	3	15	21	9	5										1.7688
2711.5	1.786	2820	52					5	9	23	8	7									1.7787
			471			10	40	105	182	85	49										1.7716
2813.1	1.824	2911	47							5	14	18	7	3							1.9130
2813.2	1.886	2912	50							3	10	19	13	5							1.9320
2813.3	1.699	2913	53							2	8	24	15	4							1.9354
2813.4	1.852	2914	52							2	5	20	19	6							1.9461
2813.5	1.731	2915	56							1	7	23	15	8	2						1.9500
2817.1	1.812	2916	45							6	10	15	11	3							1.9195
2817.2	1.763	2917	47							4	10	17	12	4							1.9271
2817.3	1.749	2918	41							3	8	16	10	4							1.9299
2817.4	1.674	2919	45						1	4	5	19	13	3							1.9283
2817.5	1.875	2920	46							5	4	17	16	4							1.9358
			482							1	35	81	188	131	44	2					1.9323
2912.1	1.874	3111	52										3	6	12	19	7	3	2		2.1115
2912.2	2.032	3112	46											5	9	18	10	3	—	1	2.1261
2912.3	1.986	3113	41										1	—	8	16	10	5	1		2.1396
2912.4	1.935	3114	39											2	5	19	8	4	—	1	2.1391
2912.5	1.825	3115	56										4	6	10	20	7	5	2	2	2.1277
2920.1	1.997	3116	54										2	2	8	20	9	7	4	2	2.1473
2920.2	2.041	3117	57									1	1	5	9	19	11	4	5	2	2.1390
2920.3	1.893	3118	58									2	3	1	9	21	10	7	2	4	2.1413
2920.4	2.034	3119	45										3	1	6	17	12	4	2		2.1350
2920.5	1.945	3120	41											3	7	18	9	3	—	1	2.1323
			489									3	17	31	82	187	93	45	18	13	2.1334
3114.1	2.136	3211	53										1	3	11	21	13	4			2.1259
3114.2	2.094	3212	58										2	2	13	20	12	7	2		2.1327
3114.3	2.037	3213	47									1	3	1	9	17	11	2	3		2.1250
3114.4	2.336	3214	51									2	2	3	8	22	10	1	2	1	2.1191
3114.5	2.295	3215	50									1	3	2	10	19	9	3	3		2.1220
3117.1	2.168	3216	54										3	5	7	21	11	3	4		2.1277
3117.2	2.075	3217	57										2	2	10	22	13	6	2		2.1346
3117.3	1.934	3218	43										1	5	9	17	7	2	1	1	2.1203
3117.4	2.238	3219	52										2	4	11	22	9	1	2	1	2.1212
3117.5	2.329	3220	51										3	7	8	18	10	3	2		2.1162
			516									4	22	34	96	199	105	32	21	3	2.1248

TABLE 7. INDIVIDUAL MEANS OF PODLENGTHS IN GROUP III (D_2 - D_4 , 1927-1929)
(Class 22 = 17.51-17.75; class 31 = 19.76-20.00)

Parent	Mean of parent	No.	number of plants	Class											M ± σ
				22	23	24	25	26	27	28	29	30	31		
2624.1	19.11	2713	69		3	1	8	22	17	6	7	3	2	18.817 ± 0.426	
2624.2	18.34	2714	52		3	2	9	16	13	5	1		1	18.693 ± 0.363	
2624.3	18.19	2715	65	3	7	11	24	13	1	4	2			18.379 ± 0.381	
			186	3	13	14	41	53	31	15	10	3	3	18.662 ± 0.374	
2713.1	18.75	2821	46		5	6	9	12	7	3	4			18.565 ± 0.421	
2713.2	18.37	2822	57	1	3	5	8	15	13	6	4	2		18.686 ± 0.435	
2713.3	18.12	2823	58			3	7	13	16	9	6	3	1	18.866 ± 0.394	
2713.4	17.93	2824	63		4	7	9	12	17	6	5	1		18.693 ± 0.421	
2713.5	19.54	2825	48		2	3	10	16	9	7	1			18.646 ± 0.334	
2715.1	18.81	2826	53		1	4	9	21	11	5	2			18.658 ± 0.311	
2715.2	18.93	2827	57		2	5	13	19	10	4	3	1		18.633 ± 0.363	
2715.3	17.62	2828	49	1	1	3	11	18	12	1	2			18.615 ± 0.323	
2715.4	18.15	2829	52			2	8	20	13	5	3	1		18.740 ± 0.315	
2715.5	19.36	2830	55		1	3	5	22	15	7	1	1		18.725 ± 0.311	
			538	2	19	41	89	166	123	55	31	9	1	18.696 ± 0.376	
2823.1	18.21	2921	51		1	7	8	18	13	4				18.606 ± 0.301	
2823.2	19.63	2922	54		2	3	9	21	11	7	1			18.657 ± 0.315	
2823.3	19.18	2923	59		1	5	7	22	13	6	2	3		18.722 ± 0.371	
2823.4	19.41	2924	43	1	2	4	8	15	9	2	2			18.584 ± 0.365	
2823.5	18.73	2925	58		2	5	11	20	11	6	2	1		18.651 ± 0.355	
2829.1	19.23	2926	47			2	9	19	8	5	3	1		18.721 ± 0.328	
2829.2	18.51	2927	60		2	3	11	22	10	7	3	1	1	18.699 ± 0.364	
2829.3	18.07	2928	43		1	1	7	16	8	4	5	1		18.758 ± 0.367	
2829.4	18.83	2929	52			3	9	21	11	6	2			18.692 ± 0.291	
2829.5	19.61	2930	55		1	5	11	19	14	2	3			18.638 ± 0.318	
			522	1	12	38	90	193	108	49	23	7	1	18.674 ± 0.345	

TABLE 9. INDIVIDUAL MEANS OF PODLENGTHS IN RECIPROCAL C

Parent	Mean of parent	Generation	No	number of plants	Class					
					4	5	6	7	8	9 10
2701.1 × 2715.4	12 29 × 18 15	F ₁	2831	7	1	1	2	2	1	
2831.1	13 77	F ₂	3001	54			1	5	14	21 8
2831.2	13 53		3002	57			5	7	12	18 10
2831.3	13 32		3003	56			1	3	13	20 15
2831.4	13 83		3004	60			3	8	11	21 13
2831.5	14 12		3005	50				5	9	22 10
				277			10	28	59	102 56
3002.1	14 41	F ₃	3121	51						
3002.2	14 88		3122	48						
3002.3	14 13		3123	60						1
3004.1	13 93		3124	57						1
3004.2	14 61		3125	53						2
				269						4
3123.1	15 57	F ₄	3311	60						
3123.2	15 38		3312	58						
3125.1	15 43		3313	57						
3125.2	14 65		3314	54						
3125.3	15 79		3315	53						
				282						
3311.1	16 53	F ₅	3411	49						
3312.1	16 39		3412	53						
3312.2	15 86		3413	51						
3314.1	16 31		3414	59						
3314.2	16.58		3415	58						
				270						
2715.4 × 2701.1	18 15 × 12 29	F ₁	2832	5						
2832.1	18.91	F ₂	3006	56						
2832.2	18 41		3007	51						
2832.3	18.29		3008	59						
2832.4	19 13		3009	53						
2832.5	18.65		3010	54						
				273						

SUP I / GROUP III (Class 4 = 13.01-13.25; class 30 = 19.51-19.75)

14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	M ± σ
																	13 629 ± 0 303
																	14 333 ± 0 284
																	14 292 ± 0 361
																	14 379 ± 0 269
																	14 321 ± 0 331
																	14 375 ± 0 274
																	14 339 ± 0 309
10	5	1															15 419 ± 0.261
11	3	4															15 453 ± 0.311
14	7	2															15 421 ± 0.318
13	5	3															15 402 ± 0.336
9	4	3															15 375 ± 0.343
57	24	13															15 412 ± 0.280
2	3	13	24	11	6	1											16 379 ± 0.301
4	7	9	21	12	5												16 319 ± 0.332
2	5	13	17	15	4	1											16 362 ± 0.307
3	5	8	20	11	7												16 366 ± 0.330
1	4	11	22	8	6	1											16 370 ± 0.301
12	24	54	104	57	28	3											16 361 ± 0.317
				1	5	9	17	11	4	2							17.390 ± 0.325
					5	7	21	13	6	1							17 427 ± 0.289
				2	3	10	24	7	3	2							17 360 ± 0.307
				1	8	9	22	11	4	4							17 387 ± 0.346
				2	5	10	19	16	6								17.383 ± 0.308
				6	26	45	103	58	23	9							17 390 ± 0.317
											2	1	1	1			18 678 ± 0.324
									2	3	10	19	16	3	2	1	18 669 ± 0.334
									1	2	12	20	9	7			18 645 ± 0.284
									2	5	14	23	12	3			18 574 ± 0.279
										3	13	17	15	4		1	18.662 ± 0.288
									1	7	9	22	11	2	2		18.602 ± 0.309
									6	20	58	101	63	19	4	2	18.629 ± 0.307

TABLE 10. INDIVIDUAL MEANS OF PODWIDTHS IN RECIPROCAL C

Parent	Mean of parent	Generation	No.	number of plants	Class		4
					2	3	
2701.1 × 2715.4	1.542 × 2.241	F ₁	2831	7	1	2	1
2831.1	1.524	F ₂	3001	54	1	3	11
2831.2	1.619		3002	57		4	14
2831.3	1.673		3003	56	1	5	15
2831.4	1.691		3004	60	2	8	9
2831.5	1.483		3005	50	1	5	8
				277		5	25
3002.1	1.579	F ₃	3121	51		1	3
3002.2	1.536		3122	48		1	6
3002.3	1.723		3123	60		2	5
3004.1	1.691		3124	57		2	4
3004.2	1.584		3125	53		3	3
				269			9
3123.1	1.823	F ₄	3311	60			
3123.2	1.745		3312	58			
3125.1	1.614		3313	57			
3125.2	1.721		3314	54			
3125.3	1.584		3315	53			
				282			
3311.1	1.824	F ₅	3411	49			
3312.1	1.734		3412	53			
3312.2	1.685		3413	51			
3314.1	1.773		3414	59			
3314.2	1.659		3415	58			
				270			
2715.3 × 2701.1	2.241 × 1.542	F ₁	2832	5			
2832.1	2.176	F ₂	3006	56			
2832.2	2.212		3007	51			
2832.3	2.093		3008	59			
2832.4	2.158		3009	53			
2832.5	2.067		3010	54			
				273			

UP I : GROUP III (Class 2 = 1.46-1.50; class 18 = 2.25-2.30)

8	9	10	11	12	13	14	15	16	17	18	M \pm σ
											1.5866 \pm 0.0720
4											1.6389 \pm 0.0690
1											1.6258 \pm 0.0550
1											1.6196 \pm 0.0602
2											1.6200 \pm 0.0651
1											1.6270 \pm 0.0608
9											1.6261 \pm 0.0624
5											1.6809 \pm 0.0574
3	2										1.6698 \pm 0.0639
6	2										1.6775 \pm 0.0648
5											1.6706 \pm 0.0587
3	1										1.6654 \pm 0.0622
22	5										1.6729 \pm 0.0617
19	14	3									1.7625 \pm 0.0596
22	10	7	1								1.7759 \pm 0.0639
20	13	4	2								1.7741 \pm 0.0652
16	14	1	2								1.7676 \pm 0.0641
21	11	6	1								1.7788 \pm 0.0628
98	62	21	6								1.7716 \pm 0.0634
	3	10	19	9	7	1					1.9352 \pm 0.0580
2	5	11	18	12	5						1.9203 \pm 0.0618
1	4	10	23	8	3	2					1.9241 \pm 0.0555
	5	12	25	10	6	1					1.9275 \pm 0.0565
1	4	9	21	13	7	3					1.9388 \pm 0.0648
4	21	52	106	52	28	7					1.9293 \pm 0.0606
						2		2	1		2.1414 \pm 0.0535
				1	6	5	22	13	7	2	2.1366 \pm 0.0648
				7	10	24	9	9	1		2.1123 \pm 0.0483
				3	5	12	19	15	4	1	2.1208 \pm 0.0647
				1	4	13	21	11	3		2.1184 \pm 0.0532
				2	5	9	20	13	3	2	2.1250 \pm 0.0645
				7	27	49	106	61	18	5	2.1228 \pm 0.0603

TABLE 11. INDIVIDUAL MEANS OF PODLENGTHS IN RECIPROCAL C

Parent	Mean of parent	Generation	No.	number of plants	Class						
					4	5	6	7	8	9	10
2701 1 × 2708 2	12 29 × 14.83	F ₁	2833	11	2	1	3	3	2		
2833 1 2833 2 2833 3 2833 4 2833.5	13 62 13 04 14 13 13 73 13 79	F ₂	3011 3012 3013 3014 3015	53 58 55 57 59				3 1 2 4 3	11 7 5 13 3	22 20 17 23 21	10 13 14 11 15
				282				6	22	58	103
3011 1 3011 2 3011 3 3011 4 3014 1	13 84 15.12 14 53 14 37 14 81	F ₃	3126 3127 3128 3129 3130	49 55 56 51 58							1 2 1 1
				269							5
3127 1 3128 1 3128 2 3128 3 3130 1	14 93 14 72 15 53 15 21 16 11	F ₄	3316 3317 3318 3319 3320	57 48 60 54 54							
				273							
3317 1 3317.2 3317 3 3319 1 3319.2	16 21 15 83 16 78 17.19 16 15	F ₅	3416 3417 3418 3419 3420	53 46 58 59 55							
				271							
2708 2 × 2701.1	14.83 × 12.29	F ₁	2834	10							

TABLE 11 (Continued)

Parent	Mean of parent	Generation	No.	number of plants	Class						
					4	5	6	7	8	9	10
2834.1	16.41	F ₂	3016	45							
2834.2	15.81		3017	57							
2834.3	16.23		3018	56							
2834.4	15.93		3019	59							
2834.5	16.84		3020	54							
				271							
3018.1	16.93	F ₃	3221	58							
3018.2	16.77		3222	56							
3019.1	17.41		3223	42							
3019.2	17.19		3224	53							
3019.3	17.74		3225	51							
				260							
3223.1	18.61	F ₄	3321	56							
3223.2	18.19		3322	53							
3223.3	18.81		3323	56							
3223.4	18.43		3324	60							
3223.5	18.35		3325	58							
				283							

14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	M \pm σ
					3	11	15	14	2								17.380 \pm 0.250
				2	4	13	23	7	6	2							17.366 \pm 0.324
				1	5	14	20	9	7								17.357 \pm 0.273
				3	7	12	24	10	3								17.294 \pm 0.307
				1	4	15	19	11	4								17.343 \pm 0.276
				7	23	65	101	51	22	2							17.347 \pm 0.293
									2	1	13	21	16	3	2		18.655 \pm 0.294
									3	5	9	18	10	7	3	1	18.675 \pm 0.374
									1	2	8	15	12	4			18.656 \pm 0.294
									2	6	9	20	11	3	2		18.606 \pm 0.329
										4	10	19	10	5	3		18.679 \pm 0.314
									8	18	49	93	59	22	10		18.652 \pm 0.326
										3	9	22	13	6	3		18.705 \pm 0.297
									1	4	10	17	14	5	2		18.667 \pm 0.318
									2	3	7	23	9	7	5		18.706 \pm 0.364
									3	2	10	24	11	8	2		18.676 \pm 0.330
									2	2	9	19	13	7	5	1	18.741 \pm 0.366
									8	14	45	105	60	33	17	1	18.690 \pm 0.337

TABLE 12. INDIVIDUAL MEANS OF PODWIDTHS IN RECIPROCAL CROS

Parent	Mean of parent	Generation	No.	number of plants	Class			
					2	3	4	5
2701.1 × 2708.2	1.542 × 1.595	F ₁	2833	11	1	3	3	4
2833.1	1.584	F ₂	3011	53		4	11	20
2833.2	1.472		3012	58	2	5	10	23
2833.3	1.633		3013	55	1	5	7	19
2833.4	1.513		3014	57		2	13	21
2833.5	1.591		3015	59	1	3	11	21
				282	4	19	52	104
3011.1	1.684	F ₃	3126	49			2	9
3011.2	1.538		3127	55		1	3	11
3011.3	1.617		3128	56			5	13
3011.4	1.726		3129	51			3	6
3014.1	1.559		3130	58		2	2	12
				269		3	15	51
3127.1	1.591	F ₄	3316	57				
3128.1	1.762		3317	48				1
3128.2	1.593		3318	60				1
3128.3	1.647		3319	54				
3130.1	1.521		3320	54				
				273				2
3317.1	1.681	F ₅	3416	53				
3317.2	1.823		3417	46				
3317.3	1.714		3418	58				
3319.1	1.747		3419	59				
3319.2	1.823		3420	55				
				271				
2708.2 × 2701.1	1.595 × 1.542	F ₁	2834	10				

GROUP I \times GROUP II (Class 2 = 1.46-1.50; class 18 = 2.25-2.30)

7	8	9	10	11	12	13	14	15	16	17	18	M \pm σ
												1 5705 \pm 0 0498
5	1											1 6307 \pm 0.0563
7	2											1 6276 \pm 0 0661
4	3											1 6368 \pm 0 0646
6	2											1 6373 \pm 0 0564
7	1											1 6352 \pm 0 0588
29	9											1 6335 \pm 0 0607
14	3	1										1 6852 \pm 0 0505
15	6	2										1 6868 \pm 0 0625
10	5	1										1 6750 \pm 0 0572
12	3	4										1 6926 \pm 0.0592
15	4	2										1 6810 \pm 0.0610
66	21	10										1 6839 \pm 0 0586
10	21	13	7	2								1 7882 \pm 0 0596
11	20	9	3	2								1 7781 \pm 0 0590
9	24	11	6	3								1 7816 \pm 0 0651
12	21	9	3	4								1 7796 \pm 0 0640
13	19	12	4	2								1.7797 \pm 0 0587
55	105	54	23	13								1 7811 \pm 0.0618
	1	1	14	25	9	3						1 9212 \pm 0 0475
	1	4	10	13	12	5	1					1 9293 \pm 0 0643
		5	9	24	11	4	5					1 9379 \pm 0 0648
	3	3	13	19	15	4	2					1 9258 \pm 0 0654
	2	3	10	20	13	5	2					1 9314 \pm 0 0640
	7	16	56	101	60	21	10					1 9292 \pm 0 0618
2	2	3	2									1 7900 \pm 0 0635

TABLE 12 (Continued)

Parent	Mean of parent	Generation	No.	number of plants	Class				
					2	3	4	5	6
2834.1	1.776	F ₂	3016	45					
2834.2	1.683		3017	57					
2834.3	1.865		3018	56					
2834.4	1.741		3019	59					
2834.5	1.823		3020	54					
				271					
3018.1	1.971	F ₃	3221	58					
3018.2	2.032		3222	56					
3019.1	1.932		3223	42					
3019.2	1.834		3224	53					
3019.3	2.017		3225	51					
				260					
3323.1	2.193	F ₄	3321	56					
3323.2	2.214		3322	53					
3323.3	2.064		3323	56					
3323.4	2.136		3324	60					
3323.5	2.181		3325	58					
				283					

8	9	10	11	12	13	14	15	16	17	18	$M \pm \sigma$
	3	13	14	11	3	1					$1\ 9260 \pm 0\ 0562$
2	5	7	24	10	6	3					$1\ 9320 \pm 0\ 0678$
1	4	11	21	8	7	4					$1\ 9357 \pm 0.0686$
1	5	12	22	14	3	2					$1\ 9258 \pm 0\ 0594$
	3	9	23	10	6	3					$1\ 9388 \pm 0\ 0600$
4	20	52	104	53	25	13					$1.9320 \pm 0\ 0630$
					3	7	22	13	9	4	$2\ 1508 \pm 0\ 0619$
				1	5	10	17	14	6	3	$2\ 1357 \pm 0\ 0652$
					4	5	19	12	2		$2\ 1286 \pm 0.0499$
					3	8	20	10	7	5	$2\ 1485 \pm 0\ 0651$
					3	11	18	15	3	1	$2\ 1319 \pm 0\ 0533$
				1	18	41	96	64	27	13	$2\ 1393 \pm 0\ 0611$
					3	9	22	13	8	1	$2.1402 \pm 0\ 0558$
				2	2	10	18	12	7	2	$2.1363 \pm 0\ 0617$
				1	2	8	20	15	9	1	$2\ 1437 \pm 0\ 0585$
					3	11	24	13	6	3	$2\ 1392 \pm 0\ 0587$
				4	4	10	17	16	5	2	$2\ 1267 \pm 0\ 0676$
				7	14	48	101	69	35	9	2.1377 ± 0.0624

TABLE 8. INDIVIDUAL MEANS OF PODWIDTHS IN GROUP III (D_2 - D_4 , 1927-1929)

(Class 11 = 1.91-1.95; class 19 = 2.31-2.35)

Parent	Mean of parent	No.	number of plants	Class										M ± σ
				11	12	13	14	15	16	17	18	19		
2624.1	2.22	2713	69		7	4	17	24	10	5	—	2	2.1105 ± 0.0738	
2624.2	2.08	2714	52		4	8	9	12	11	5	3	2	2.1173 ± 0.0802	
2624.3	2.17	2715	65	2	2	10	21	18	9	1	2	2	2.0958 ± 0.0679	
			186	2	13	22	47	54	30	11	5	2	2.1081 ± 0.0746	
2713.1	1.984	2821	46		3	6	8	13	7	5	4		2.1254 ± 0.0815	
2713.2	2.137	2822	57	3	2	2	16	19	9	5	1		2.1113 ± 0.0736	
2713.3	2.215	2823	58		2	5	13	21	10	3	4		2.1248 ± 0.0685	
2713.4	2.036	2824	63			3	18	25	11	4	2		2.1258 ± 0.0546	
2713.5	2.333	2825	48			4	5	17	10	8	2	2	2.1530 ± 0.0713	
2715.1	2.273	2826	53	2	4	3	11	18	9	4	2		2.1119 ± 0.0784	
2715.2	2.185	2827	57		3	5	13	22	10	3	1		2.1136 ± 0.0632	
2715.3	2.129	2828	49		1	2	15	17	12	1	1		2.1199 ± 0.0537	
2715.4	2.241	2829	52	1	—	5	12	22	11	—	1		2.1135 ± 0.0516	
2715.5	1.948	2830	55		2	4	13	20	9	3	3	1	2.1259 ± 0.0677	
			538	6	17	39	124	194	98	36	21	3	2.1221 ± 0.0683	
2823.1	2.030	2921	51		2	5	9	17	12	5	1		2.1250 ± 0.0656	
2823.2	2.279	2922	54		1	4	11	20	10	6	1	1	2.1315 ± 0.0660	
2823.3	2.214	2923	59	1	3	3	12	24	9	5	2		2.1199 ± 0.0693	
2823.4	2.297	2924	43	1	1	2	8	15	10	3	3		2.1320 ± 0.0719	
2823.5	2.185	2925	58		3	2	13	22	11	4	3		2.1267 ± 0.0662	
2829.1	2.125	2926	47		2	5	7	18	8	5	1	1	2.1271 ± 0.0722	
2829.2	1.039	2927	60		2	7	10	21	12	3	4	1	2.1217 ± 0.0741	
2829.3	2.164	2928	43		1	3	8	14	7	5	—		2.1250 ± 0.0571	
2829.4	2.987	2929	52		2	3	11	20	11	4	1		2.1241 ± 0.0606	
2829.5	2.108	2930	55		2	2	10	22	12	6	1		2.1314 ± 0.0603	
			522	2	19	36	99	198	102	46	17	3	2.1270 ± 0.0663	

TABLE 13. INDIVIDUAL MEANS OF PODLENGTHS IN RECIPROCAL CROSSES

GROUP II \times GROUP III

(Class 15 = 15.76-16.00; class 30 = 19.51-19.75)

parent	Mean of parent	Generation	No.	number of plants	Class																M \pm σ		
					15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30			
708.2 \times 715.4	14.83 \times 18.15	F ₁	2835	8	1	1	3	3														16.375 \pm 0.250	
835.1	16.45	F ₂	3021	43					1	6	19	13	8	1								17.500 \pm 0.260	
835.2	15.83		3022	59						4	9	24	14	5	3							17.442 \pm 0.294	
835.3	16.31		3023	53					1	4	8	20	11	7	2							17.432 \pm 0.321	
835.4	16.62		3024	60					1	5	7	21	15	9	2							17.454 \pm 0.321	
835.5	16.20		3025	49					4	6	17	13	6	3								17.477 \pm 0.315	
				269					2	18	36	10	66	35	11							17.459 \pm 0.305	
3022.1	16.85	F ₃	3226	51											2	6	17	15	8	3		18.772 \pm 0.293	
3022.2	17.79		3227	50											1	3	5	21	11	7	1	18.715 \pm 0.323	
3022.3	17.21		3228	60											1	7	10	23	10	5	2	18.654 \pm 0.363	
3024.1	17.13		3229	56												2	9	19	16	7	5	18.763 \pm 0.308	
3024.2	17.85		3230	53												2	7	20	13	6	4	18.765 \pm 0.320	
				272											2	16	37	100	65	33	15	4	18.733 \pm 0.327
3227.1	18.47	F ₁	3326	55											2	6	22	13	7	4	1	18.775 \pm 0.315	
3227.2	18.16		3327	51											1	3	7	17	12	5	5	18.737 \pm 0.330	
3227.3	18.83		3328	47											2	2	5	20	9	5	4	18.710 \pm 0.347	
3227.4	19.12		3329	59												3	9	23	11	8	3	21.748 \pm 0.329	
3230.1	19.41		3330	54											2	1	8	21	15	7	2	3	18.745 \pm 0.375
				266											5	11	35	103	55	33	18	6	18.744 \pm 0.346
2715.4 \times 2708.2	18.15 \times 14.83	F ₁	2836	9												3	2	3	1			18.680 \pm 0.258	
2836.1	18.41	F ₂	3026	58											1	2	7	24	13	9	2		18.724 \pm 0.297
2836.2	19.14		3027	51											2	5	22	10	7	3	2		21.782 \pm 0.332
2836.3	18.27		3028	56											3	7	25	12	5	4			18.727 \pm 0.294
2836.4	18.32		3029	59											2	1	6	21	13	6	3	2	18.765 \pm 0.342
2836.5	18.51		3030	50											3	2	19	13	8	4	1		18.810 \pm 0.324
				274											3	11	27	111	66	35	16	5	18.758 \pm 0.321

TABLE 14. INDIVIDUAL MEANS OF PODWIDTHS IN RECIPROCAL CROSSES

GROUP II \times GROUP III

(Class 7 = 1.70-1.75; class 18 = 2.25-2.30).

Parent	Mean of parent	Generation	No.	number of plants	Class 7	8	9	10	11	12	13	14	15	16	17	18	M \pm σ
2708.2 \times 2715.4	1.595 \times 2.241	F ₁	2835	8	2	2	3	1									1.7937 \pm 0.0496
2835.1	1.834	F ₂	3021	48			4	6	18	11	7	2					1.9427 \pm 0.0616
2835.2	1.723		3022	59			3	10	23	14	5	4					1.9419 \pm 0.0601
2835.3	1.814		3023	53			4	9	17	13	6	3	1				1.9448 \pm 0.0675
2835.4	1.767		3024	60			3	11	22	10	7	5	2				1.9500 \pm 0.0716
2835.5	1.856		3025	49			3	8	18	11	4	4	1				1.9464 \pm 0.0677
				269			17	44	98	59	29	18	4				1.9453 \pm 0.0660
3022.1	1.946	F ₂	3226	51							5	6	21	9	8	2	2.1397 \pm 0.0582
3022.2	1.873		3227	50							3	9	16	16	5	1	2.1390 \pm 0.0557
3022.3	1.993		3228	60						1	3	10	23	14	6	3	2.1383 \pm 0.0618
3024.1	2.014		3229	58						2	5	11	19	15	4	2	2.1267 \pm 0.0650
3024.2	1.923		3230	53						6	12	17	13	3	2		2.1259 \pm 0.0603
				272						3	22	48	96	67	26	10	2.1342 \pm 0.0618
3227.1	2.146	F ₂	3326	55							4	11	19	13	6	2	2.1359 \pm 0.0601
3227.2	2.012		3327	51						1	4	9	16	11	7	3	2.1387 \pm 0.0686
3227.3	2.164		3328	47						1	3	8	20	9	5	1	2.1303 \pm 0.0594
3227.4	2.091		3329	59						1	5	12	24	13	4		2.1216 \pm 0.0543
3230.1	2.103		3330	54						2	4	10	21	10	6	1	2.1259 \pm 0.0628
				266						5	20	50	100	56	28	7	2.1303 \pm 0.0615
2715.4 \times 2708.2	2.241 \times 1.595	F ₁	2836	9								3	1	3	2		2.1472 \pm 0.0583
2836.1	2.241	F ₂	3026	58						1	3	11	24	13	6		2.1293 \pm 0.0544
2836.2	2.087		3027	51						3	9	20	15	3	1		2.1338 \pm 0.0521
2836.3	2.146		3028	56						2	12	23	14	5			2.1321 \pm 0.0484
2836.4	2.123		3029	59						3	4	9	24	11	7	1	2.1267 \pm 0.0651
2836.5	2.219		3030	50						3	10	21	9	6	1		2.1330 \pm 0.0560
				274						4	15	51	112	62	27	3	2.1308 \pm 0.0558

STUDIES IN VIOLA, 1: THE CYTOLOGY OF A NATURALLY-OCCURRING POPULATION OF HYBRIDS BETWEEN *VIOLA TRICOLOR* L. AND *VIOLA LUTEA* HUDS.

by

PHILIP G. FOTHERGILL, Ph.D.

University College of Swansea

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1. INTRODUCTION

On the North bank of the River Tyne between two points, one almost opposite Bywell Hall and the other opposite the ruins of Bywell Castle, there is situated a large population of hybrids between *Viola tricolor* L. and *V. lutea* HUDS. Bywell is near to Stocksfield in the County of Northumberland. The length of the area

covered by the population is, roughly, about one-third of a mile, while the average breadth is approximately seventy yards. On one side of the area is the shingle bank of the Tyne while the opposite side is bounded by a meadow.

It was thought that a hybrid population of this type could be used to discover the survival value of various cytological types. With this end in view the chromosome numbers of the plants, their meiotic regularity, or otherwise, and their chromosomal constitution have been studied. By considering the results in relation to the possibilities of chromosomal combinations and numbers it was hoped to be able to indicate the present status of the population in cytological terms.

The genus *Viola* has been studied both genetically and cytologically, notably by J. CLAUSEN. The investigations of hybrids of *Viola* were started by KRISTOFFERSON (1914, 1916, 1923), but they were confined to the genetics. CLAUSEN (1926) first combined the cytology with the genetics of the hybrids. The study of the somatic chromosomes of species in the genus was first carried out by MIYAJI (1913, 1927) and later by HEILBORN (1926) and GERSHOY (1928). The morphology and taxonomy of our British Pansies have been studied in detail by DRABBLE (1909 etc.).

2. MATERIALS AND METHODS

In the summer of 1933 flower buds were fixed and seeds obtained from a plant of the wild population named V.B.15. At the same time buds and seeds were taken from two other plants (V.B.18. and V.B.19). These two plants had originally formed part of the wild population but had been transplanted to a nearby garden separated from the population by a rather steep grassy slope and a field. The seeds were sown almost immediately. There were only six seeds of V.B.19., however, and none of them germinated. As regards the other two sets of seeds, germination was very good. They germinated shortly after Christmas 1934. When the seedlings were at a suitable stage they were planted out separately, and later, root tips were taken. The progeny thus obtained were named V.B.18.1., V.B.18.2., etc. and V.B.15.1., V.B.15.2., etc.

Just before Easter 1934 a selection of small plants was obtained

from the garden mentioned above. Presumably these were the progeny of open pollination of V.B.18. and V.B.19. They were named V.B.25. . . . V.B.67. Root tips of these also were taken.

In the summer of 1934 plants were chosen at random from among the wild population growing on the banks of the Tyne at Bywell. Flower buds from these were fixed. These plants were named V.A., V.B.1., V.C. . . . V.U., V.V. Thus material for reduction divisions was taken from 22 random samples of the wild population.

Root tips were fixed in LANGLET's modification of NAWASCHIN's fixative for 24 hours. Sections were cut at 8-10 μ and stained in iron-alum haematoxylin overnight. Flower buds were first fixed in CARNOY's fluid for less than one minute and were then put in LANGLET's fixative as above. Sections of these were cut at 14 μ , mordanted in alcoholic iodine for $\frac{3}{4}$ - 1 hour, rinsed in water, stained for 1 hour in aqueous gentian violet, differentiated in the alcoholic iodine and in a mixture of clove oil and xylol in equal parts, and finally mounted in Canada balsam.

Drawings were made using an Abbé camera lucida, 18 eyepiece and a Leitz $\frac{1}{12}$ in. oil immersion objective. The magnification in all cases is 4050 and reduced in reproduction to 2025.

3. MORPHOLOGICAL DESCRIPTIONS OF THE PLANTS

A detailed analysis of the morphology of each plant of the wild population was made and the characters were tabulated. In the descriptions the letter L. was placed after a character whenever it could definitely be said to agree with that found in *Viola lutea*. Similarly the letter T. indicated that the feature in question was characteristic of *V. tricolor*. At the end of each description the numbers of characters thus marked were added up in the hope that it would indicate, in a concise way, the parent which the hybrid most resembled. The results are shown in Table 1. In this table if any plant had twice, or more than twice, as many characters of one of the original parents than of the other, it was said to resemble that parent; otherwise it was described as intermediate between the two.

TABLE 1. MORPHOLOGICAL FORMULAE OF THE WILD HYBRID PLANTS INDICATING THEIR PHENOTYPIC EXPRESSION IN RELATION TO THE ULTIMATE PARENTS

Plant	Formula	Phenotype in relation to parent		
		Lutea-like	Intermediate	Tricolor-like
V.A.	11L 5T	L	—	—
V.B.1.	7L 6T	—	I	—
V.C.	7L 6T	—	I	—
V.D.	8L 8T	—	I	—
V.E.	13L 3T	L	—	—
V.F.	10L 6T	—	I	—
V.G.	10L 8T	—	I	—
V.H.	14L 5T	L	—	—
V.I.	10L 6T	—	I	—
V.J.	10L 7T	—	I	—
V.K.	5L 8T	—	I	—
V.L.	8L 7T	—	I	—
V.M.	13L 3T	L	—	—
V.N.	12L 3T	L	—	—
V.O.	11L 2T	L	—	—
V.P.	9L 7T	—	I	—
V.Q.	11L 3T	L	—	—
V.R.	10L 4T	L	—	—
V.S.	6L 6T	—	I	—
V.T.	9L 4T	L	—	—
V.U.	7L 8T	—	I	—
V.V.	10L 5T	L	—	—

Thus, while there is a good deal of variation of characters in that many combinations of parental features are possible, the main trend of the plants is either to be (1) intermediate between *Viola tricolor* and *V. lutea* or (2) to resemble *V. lutea* to a much greater extent than *tricolor*. The formulae show that 45.5% of the random sample resemble *V. lutea* to a great extent, while 54.5% are more or less intermediate. In only two cases (V.K. and V.U.) is the sum of the *tricolor*

characters greater than that of the *lutea* characters, but even these plants are better described as intermediate.

These results are in accordance with expectations, for the Garden Pansies are regarded as having originated from a *V. tricolor* \times *V. lutea* cross (WITTROCK, 1897) and they resemble *lutea* more than *tricolor*.

The plants raised from seed of V.B.18., and V.B.15., and the mixed seedlings from the garden were also subjected to detailed morphological examination. These were the plants V.B.18.1. to V.B.18.19., V.B.15.1. to V.B.15.55., and V.B.25. to V.B.67. It was also seen that all these plants possessed characters of both species and in almost any combination. Neither of the original species was obtained back in its entirety although the plants, as a whole, tended to resemble *V. lutea* much more than *V. tricolor*.

4. MEIOSIS IN THE WILD POPULATION

Table 4 summarises the meiotic conditions and gives the somatic chromosome number of some of the plants of the wild population.

The method of fixation proved on the whole satisfactory. In prophase stages the chromosomes are distributed evenly throughout the nucleus, sometimes, however, a slight contraction of the chromatin was noticed. This contraction is not regular and is probably due to fixation. Owing to the number of chromosomes present prophase is not a suitable stage for a detailed examination of them. The nucleolus is large and sometimes curiously shaped and there may be more than one of these structures in the nucleus of the pollen mother cell. Some of the plants showed the presence of numerous small irregularly shaped bodies; these appear as knots in the chromosome threads. The threads themselves are made up of numerous small granules or chromomeres not to be confused with these larger knots.

Contraction of the chromosome threads is not regular for at diakinesis some of the chromosomes are fully contracted with terminal chiasmata and have assumed almost a smooth spherical shape while others may be longer than broad with a jagged outline in which the chiasmata are not completely terminalised. In other diakinetik nuclei all the chromosomes contract equally to a more or

less spherical condition. At this stage the bivalents lie at the periphery of the nucleus and the nucleolus is small and round.

The regularity of the first meiotic division varies greatly in the same and in different plants. Some are very regular divisions, others very irregular, while others again show an intermediate condition.

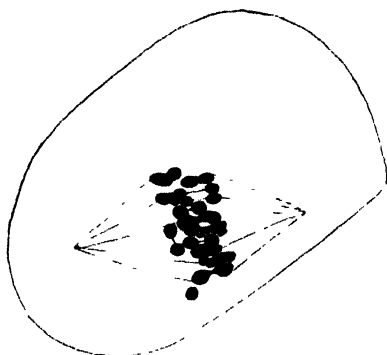


FIG. 1 Side view of 1 M in V H showing a bivalent lying parallel to the equator and a univalent just outside the spindle. All the bivalents have not been drawn in.

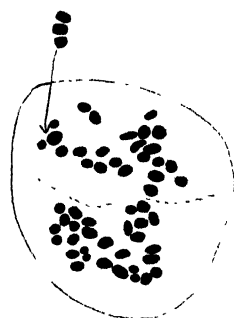


FIG. 2 Irregular orientation of the chromosomes in a polar view of 2 M in V G. Secondary association can be discerned.

Thus the chromosomes may arrange themselves at the equator of the spindler regularly but with an occasional bivalent parallel to the equator (Figure 1). Such behaviour has been observed by CLAUSEN (1931) in the cross between *Viola cornuta* and *V. elegantula*. In the extremely irregularly divisions the chromosomes lie on the equator in any haphazard fashion (figure 2) and this makes counting difficult. Univalents, bivalents, trivalents and quadrivalents may be present; only very rarely are pentavalents or sexivalents encountered. While the multivalents usually lie on the spindle, the univalents either may do so, or they may lie detached in the cytoplasm. Occasionally one or more bivalents fail to come on to the spindle (figures 3 and 4), such bodies are subsequently lost.

In nearly all plants every first metaphase showed the presence of higher associations of chromosomes than two, but bivalents were always in the great majority. The actual number of the associations often varied in one plant from pollen mother cell to pollen mother cell: this occurred especially in those plants with the more irregular

orientation of chromosomes on the spindle. The highest number of trivalents found was four and the highest number of quadrivalents

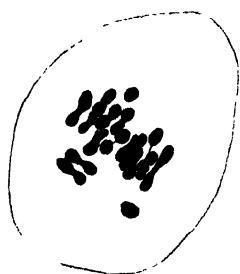


FIG. 3 Side view of 1 M in V.L. with a pair of bivalents lying parallel to the equator. Two univalents, one on each side of the equator but inside the spindle, are visible

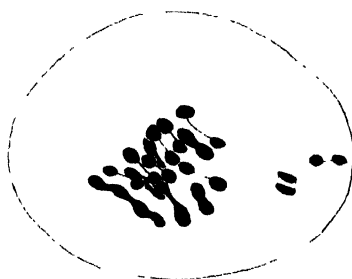


FIG. 4 Incomplete side view of 1 M of V.L. showing a quadrivalent. A pair of bivalents are lying detached in the cytoplasm.

three, mostly there were one quadrivalent and one or two trivalents present. The largest number of univalents present was ten, but,

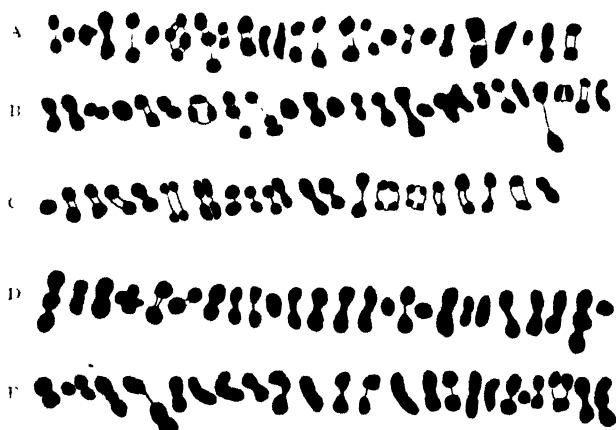


FIG. 5 Side views of 1 M. in different plants with the chromosomes drawn side by side, (A from V.E., B. from V.A., C from V.H., D from V.U., E from V.V.)

usually, there were from one to four of these (figure 5, A-E). Variation of pairing is also met with at diakinesis.

Variation such as this in the number of associations present in a hybrid is not unusual. AVERY (1930) finds a range from complete conjugation to complete lack of it in different hybrids of *Crepis leontodontoides*. She suggests that it is a reflection of previous structural change in the specific genomes of *Crepis*. LAWRENCE (1931b), on the other hand, who finds variable pairing in *Dahlia variabilis*, considers that such behaviour in hybrids is sufficiently explained by DARLINGTON's (1929) chiasma theory. CLAUSEN (1931) also finds variable pairing in many of his *Viola* hybrids.

The meiotic metaphase chromosomes of all these *Viola* hybrids are small round bodies like those of *Prunus* (DARLINGTON, 1928) or of other *Viola* species (CLAUSEN, Various papers). Most of the configurations which the trivalents formed from such chromosomes could assume, as found by DARLINGTON in *Prunus* and by MEURMAN (1929) in *Prunus laurocerasus*, have been found here. A string of three chromosomes is the most usual but V-shaped forms are not uncommon; rings are also encountered. The actual degree of association between the components of the trivalent varies and usually all three bodies are of the same size but sometimes they are unequal (figure 6).

The quadrivalent likewise assumes various forms. It may occur as a string of four chromosomes, or like two bivalents joined together



FIG. 6. 1. M, various forms of trivalents.

laterally, or as a Y-like structure. Perhaps the commonest form of quadrivalent in the hybrids under discussion is that of a ring of four chromosomes which may appear as a square or diamond-shaped body (figures 7 and 5 A-C). PHILP and HUSKINS (1931) also find



FIG. 7. 1. M., various forms of quadrivalents.

multiple associations of small chromosomes in the form of chains and rings in *Matthiola incana*. The component bodies of the quadri-

valents are usually of the same size and shape and their appearance is regular.

Occasionally, however, a size difference is noticeable between them. This is seen particularly in diakinetid rings of such plants as V.A., and V.U. (figures 9*, 10, 11), and in some trivalents as noted

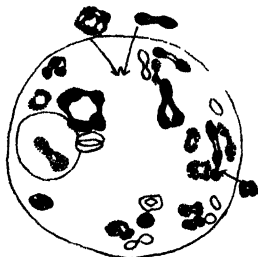


Fig. 10 Diakinesis in V.A. with one ring quadrivalent.

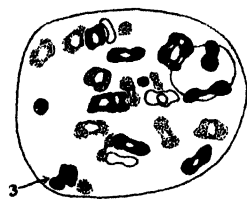


FIG. 11. Diakinesis in V.U. with one ring quadrivalent

above (figure 6 E, M, N). This may perhaps be taken as evidence of the occurrence of some structural change, such as translocation.

At anaphase the bivalents seem to separate first. The presence of univalents, which sometimes split, and of multivalents, leads to the lagging of odd chromosomes on the spindle when the other chromosomes have arrived at the poles. The amount of such lagging varies greatly from cell to cell and from plant to plant (figure 12), but about half of the pollen mother cells show it (Table 4). The actual separation of the chromosomes must in many cases be very irregular.

The chromosomes of bivalents which lie parallel to the equator may both go to the same pole. Quadrivalents usually seem to separate evenly but they may give a three and one distribution. Trivalents usually disjoin two and one but here again the middle component may, and probably does, often split. Univalents

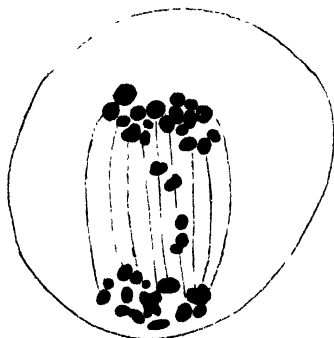


FIG. 12. Late anaphase of 1 division in V.B 18 showing lagging chromosomes. All the chromosomes at the poles have not been drawn in.

* Figures 8 and 9, see page 182

may be distributed haphazardly to the poles but in many cases they follow the bivalents on to the equator and some of them split (figure 13). Even then they may not split until they are on the way to a pole

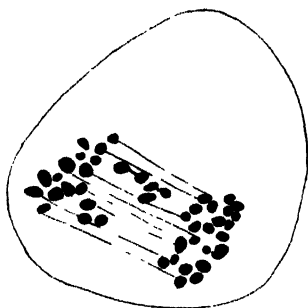


FIG 13 Late anaphase of 1 division in V U. with lagging chromosomes two of which can be seen to have split

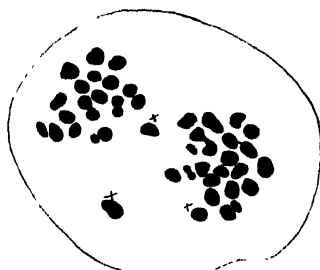


FIG 14 Polar view of 2 M. in V T with twenty chromosomes at one pole and twenty-three at the other. Three bodies (marked X) are not included in the plates, they lie in the cytoplasm

and each of the halves would be included in one of the interkinetic nuclei; others may be eliminated (figure 14). Such behaviour leads to a varying number of chromosomes at the poles, but in some cases where the first division is very irregular the same number of chromosomes may be seen at each pole as in the case of V.B.19., where all the counts showed an equal distribution of chromosomes (figure 15).

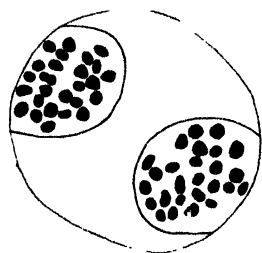


FIG. 15 Polar view of 2 M in V.B.19. with twenty-six chromosomes at each pole

Lagging univalents have been described by many workers. CLAUSEN (1931) finds them in various crosses of *Viola* species. According to PERCIVAL (1930) in various *Aegilops* and *Triticum* hybrids the univalents divide and separate to opposite poles if they lie in the equatorial zone, but if they lie near a pole the halves remain together and enter the same interkinetic nucleus as they do here. KOSTOFF (1931) considers the lagging chromosomes in some *Nicotiana* hybrids play a more important role than the other chromosomes in determining the behaviour of the following phases.

During telophase of the first division the nuclear membrane develops around each nucleus and the chromosomes gradually lose their round shape, become of rugged outline and elongate, the nucleoli then appear. In one plant, however, (V.N., figure 16), the chromosomes did not lose their shape, but remained very distinct, with a nuclear membrane that was barely discernible. Secondary association was obvious in this plant at this stage and accurate chromosome counts could be made.

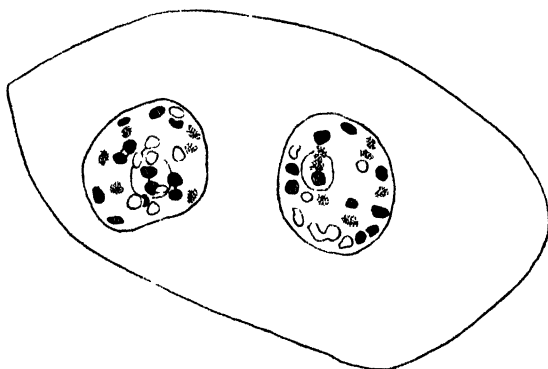


FIG. 16 Interkinesis in V N, each chromosome distinct, showing twenty-six in one nucleus, some of them connected

The second division proceeds normally, sometimes some univalents may lag behind the other chromosomes, and some of these may split. The two spindles of this division may lie at right angles or parallel to each other. On the whole tetrad formation is the rule in spite of irregular first divisions; only one case of micronuclei was observed. However, diads and triads, rarely pentads, are occasionally formed. The cells of a diad are larger than those of a triad which, in turn, are larger than those of the normal tetrad. In some cases the tetrads are misshapen and badly formed.

Secondary association is seen in most of the plants. It is obvious in polar views of the metaphases of both divisions but is seen most distinctly at the second division. In some cases chromosomes are seen to be connected at this division. Such bodies are probably the disjoined parts of multivalent associations. The postulates which LAWRENCE (1931a) lays down for secondary association are that the

pairs associated should be of equal size and similar shape and configuration, and should lie in close proximity to each other but not actually touching. Chromosomes which are secondarily paired sometimes do touch but this is probably due to a slight collapse in fixation.

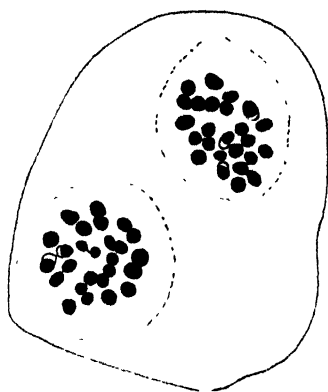


FIG 17. Polar view of 2. M. in V.H with twenty-five chromosomes at one pole and twenty-six at the other. Secondary association is very pronounced, viz. 1(4), 6(2) on one plate and 1(4), 4(2) on the other.

These requirements are fulfilled here and are often beautifully seen in side views of the metaphase (figure 5c). CLAUSEN (1927) finds many multivalents in some of his *Viola* hybrids but it was DARLINGTON and MOFFETT (1930) and LAWRENCE (1931a) who pointed out that many of CLAUSEN's drawings showed the presence of pronounced secondary association. CLAUSEN (1931) is later inclined to agree with this but guardedly points out that mere proximity is no proof of even a secondary association.

In the *Viola* hybrids under discussion the most pronounced secondary association was seen in the plant V.H. — the plant with the most regular reduction division of all those examined. The maximum amount observed was that of a second division figure with 1 group of 4 and 6 groups of 2 chromosomes (figure 17). This maximum amount occurs most frequently in plants which show the least number of multivalent primary associations.

5. MEIOSIS IN THE PLANT V.P.

The low chromosome number of this plant warrants its separate treatment. The somatic chromosome number is 26 (figure 18). All the buds of this plants were well-developed. In the early prophase the chromosomes are visible but do not fix well. Contraction of the chromosomes is not regular for at early diakinesis some of them are small and more or less spherical while the edge of others is very uneven and they

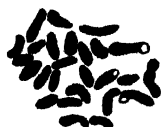


FIG. 18. Somatic plate from ovulartissue in V.P. showing twenty-six chromosomes.

are elongated. A variation in pairing was noticed; mostly there are 10 bivalents and 2 trivalents present (figure 20). Some of the bivalents seem to possess interstitial chiasmata but terminalisation is generally complete.



FIG. 20. Side view of 1. M. in V.P. with the chromosomes drawn side by side, showing two trivalents and ten bivalents

The middle component of a trivalent had split in several cases, one half going to each pole (figure 21). Univalents were found in several pollen mother cells with a corresponding decrease in the number of trivalents present (figure 24). Apparently

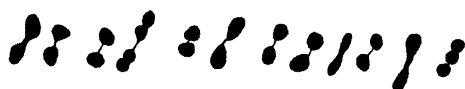


FIG. 21. Side view of 1. M. in V.P. with the chromosomes drawn side by side and showing ten bivalents and two trivalents in one of which the middle component has split

the odd members of the trivalents have an affinity for each other for several cases showed the presence of 13 bivalents (figure 22). Elimination of chromosomes was only rarely seen.

The first division is thus very regular and 13 chromosomes generally arrive at each pole (figure 19), sometimes, however, due

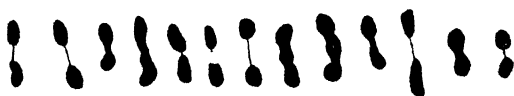


FIG. 22. Side view of 2 M. in V.P. showing thirteen bivalents drawn side by side.

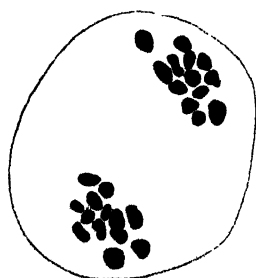


FIG. 19. Polar view of 2. M. in V.P. with thirteen chromosomes in each plate.

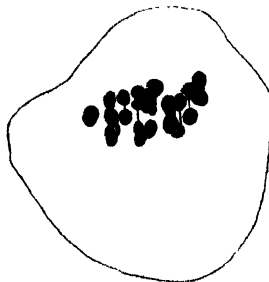


FIG. 24. Side view of 1 M. in V.P. showing two univalents, one bivalent is missing.

presumably, to the splitting of the middle component of a trivalent, 14 chromosomes are present at each pole. Secondary association is slight but evident. The maximum amount is 3 groups of 2 chromosomes. Tetrad formation is complete and regular.

6. ABNORMAL FLOWER-BUDS

Some or all of the flower-buds of V.I., V.J., V.K., V.M., V.R., and V.S., were abnormal in that they were merely empty shells consisting of sepals and petals only, or they were deficient in one or the other of the reproductive parts. Except in those that were actually empty somatic counts could sometimes be obtained from ovular tissue and the occurrence of occasional malformed anthers containing a few pollen mother cells gave some indication of their chromosomal constitution. Thus in V.I., 1 quadrivalent, 2 trivalents, and 2 univalents were seen. In V.K., a few badly-formed tetrads were present and the somatic number was found to be 48, while about 2 quadrivalents, 3 trivalents, 14 bivalents, and several univalents were present.

V.R. showed the presence of 2 quadrivalents, 2 trivalents, and 1 univalent and its somatic number was approximately 43. Finally all the flower buds of V.S. were empty shells but the somatic chromosome number was approximately 50. Another floral abnormality was seen occasionally in the development of two complete flower buds, or of two empty shells, on one peduncle.

7. CHROMOSOME NUMBER IN THE PROGENY

Somatic chromosome counts were made from root tips of the progeny of the plants V.B.18., V.B.15., and of the plants V.B. 25-67.

The somatic chromosomes of these *Viola* plants are sometimes difficult to interpret due to intertwining or overlapping ends but accurate counts could usually be made. Constrictions of the chromosomes are not easily seen but some of them are medianly constricted while others show a sub-terminal constriction; sometimes a satellited pair was noticed. While some of the larger chromosomes are bent no constant U- or J-shaped chromosomes were discernible. A detailed examination of metaphase plates was not made but it is

apparent that large, small, and probable medium length classes of chromosomes are present (figure 23).

Tables 2 and 3 show the distribution of the chromosome numbers in these plants. The possible combination of gametes in the wild population is fairly large with numbers ranging from $n = 17$ to $n = 27$, so that a wide range of chromosome numbers in the progeny is not unexpected.

Viola lutea has the somatic number $2n = 48$ (CLAUSEN, 1927, $n = 24$). The majority of the hybrid progeny group themselves around this figure. There is a big variation, however, in actual numbers. In the V.B 18. series one plant showed 27 somatic chromosomes and another 30, while in the series V.B.25-67 one plant with a number as low as 24 was found. One specimen of the wild population (V.P.) also has 26 chromosomes. These numbers are



FIG. 23 Somatic plate from a root tip of V.B 18.3. with forty-six chromosomes

TABLE 2. DISTRIBUTION OF CHROMOSOME NUMBERS

Progeny of Plant V B 18	
Chromosome numbers	27 30 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54
Number of plants	1 1 1 1 1 1 1 1 1 1 2 1 1 3 2
Progeny of Plant V B 15	
Chromosome numbers	45 46 47 48 49 50 51 52 53 54 55 56 57 58
Number of Plants	1 1 1 1 1 8 2 6 1 1 1
Plants V B 25-67	
Chromosome numbers	24 40 41 42 43 44 45 46 47 48 49 50
Number of plants	1 1 2 1 10 6 1 5 2 1

exceptions. It can be seen that a high proportion of the series V.B.

TABLE 3. TOTAL DISTRIBUT

Chromosome numbers	Progeny of Plants V.B									
	24	27	30	40	41	42	43	44	45	
Number of Plants	1	1	1	2	—	3	1	10	2	
Percentage number of Plants	1.3	1.3	1.3	2.6	—	3.9	1.3	13.0	2.6	1

25-67 have 44 as their somatic number. V.B.18. and V.B.19. were plants of the wild population that had been transferred from the natural habitat to a nearby garden, the plants V.B.25-67 were the natural progeny of these. Seeds from V.B.18. were collected and planted under ordinary hothouse conditions and among the resulting plants there is also a very high proportion (about 43%) with numbers less than $2n = 48$. Wild seedlings of a hardy plant in a garden or hothouse would probably have a much better chance of survival, at any rate, there would be less competition, than in their natural environment. Perhaps the removal of the plants V.B.18 and V.B.19. to a garden is responsible for the high proportion of low chromosome-numbered plants among their offspring. It is to be noted in this connection that, of the plants of the wild population, only a very small number have a low chromosome number and (with the exception of V.P.) these are the most irregular in their meiotic divisions. Sometimes they also possess abnormal flowers.

Thus the hybrids with low chromosome numbers (except V.P.) are cytologically unstable and are met with only under sheltered environmental conditions. Although the data available can hardly be said to be an adequate proof, it seems probable that we have here the correlation of the operation of Natural Selection and the cytological constitution: due to removal of competition, the plants less favourable to a wild habitat have had a chance to survive and to reach maturity. It seems by comparison of Tables 3 and 4 that these are the plants with chromosome numbers of $2n = 44$ or less. In nature few of them survive. Those with less than this number then tend to be eliminated. As the meiotic divisions of such plants have

F CHROMOSOME NUMBERS

B.15.; Plants V.B. 25-67.

47	48	49	50	51	52	53	54	55	56	57	58	Total
1	18	3	10	2	9	1	3	—	—	—	1	77
.3	23.4	3.9	13.0	2.6	11.7	1.3	3.9	—	—	—	1.3	

been shown to be irregular, their weakness, which leads to their extinction, may be due to a greater unbalance of chromosomes in them than in their more favourable sister and brother plants with a larger and perhaps more balanced number of chromosomes.

The central chromosome number is 48—23% of the plants possess this number. An almost equal number of plants have $2n = 46$, $2n = 50$, $2n = 44$, $2n = 52$, while the number of plants with $2n = 42$ and $2n = 54$ is equal. The percentage of plants with chromosome numbers above or below these numbers is very small. Another interesting point to be noticed in Table 3 is the very low proportion of plants with odd numbers.

8. DISCUSSION AND CONCLUSION

A. *The Hybridity of the Plants in question*

A careful detailed study of the external characters of the plants shows that they are hybrids between *Viola tricolor* L. and *V. lutea* Huds. Many of them are morphologically intermediate between these two species; many of them also possess a majority of *lutea* characters, while only a few show a majority of *tricolor* characters. Most of the plants of the wild population show an increased vigour and size of parts — a sign of hybridity. In addition, DRABBLE (1909) states that a feature of British *Viola* hybrids is the characteristic alteration of the form of the stipule. He would consider this in a plant very good evidence in itself of hybridity, even if he had not seen the

TABLE 4. SUMMARY OF THE STATE OF MEIOSIS IN THE PLANTS OF THE WILD POPULATION.

(Figures in brackets indicate the number of Chromosomes in an association).

Plant	State of Division	Associations, 1st Metaphase or diakinesis	Number of Chromosomes at 2nd. division poles	Per cent of lagging	Secondary association	Somatic Chromosome number	Pollen formation
V.B.19	very irregular	varies 1(6) 2(4) 2(3) 14(2) 4(1) usually 1(4) 2(3) 17(2) 8(1)	26-26	17%	present	52	tetrads only
V.B.18	very irregular	varies, trivalents, quadrivalents present, 1(6) found once	23 to 26	nearly every case	present	50	tetrads only
V.A.	varies	1(4) 4(3) 17(2) 4(1) occasionally 1(5)	23 to 27	slight at 2nd division	pronounced 1(3), maximum 8(2)	54	tetrads only
V.E.	regular	1(4) 1(3) 17(2) 6(1); univalents & trivalents vary correspondingly	23 to 25	50%	very small amount 5(2)	47	tetrads only
V.F.	irregular	1(4) 1(3) 17-18(2) 1(1); univalents, trivalents vary	20 to 26 majority 22	28%	pronounced maximum 8(2) present	51-53	tetrads rarely pentad
V.G.	irregular	—	26	50%	1(3), maximum 6(2)	—	diads triads tetrads pentads
V.H.	regular	1-2(4) 21-23(4) 1(1) rarely 1(3)	24 to 26	occasional	pronounced 1(3), maximum 8(2)	51	tetrads only
V.J.	Flowers mostly empty shells	probably 1(3) 13-16(2) 9-10(1)	—	—	—	39-45	Misshapen tetrads few triads
V.L.	irregular	varies, uncertain about 1(4) 2(3) 17(2) 2(1)	23 to 25	24%	pronounced 1(3), maximum 8(2)	at least 46	tetrads only
V.N.	irregular	varies, uncertain 1-3(4) 2(3), 15-19(2) 5(1)	25 to 27, at interkinesis 22 to 26	50%	pronounced 1(3), maximum 6(2)	53	—
V.O.	—	1(4) 1(3) 21(2) 1(1)	—	—	—	50	—
V.P.	regular	very slightly, mostly 2(3) 10(3) sometimes 1 or 2(1) or 13(2)	13 to 14	—	present, maximum 3(2)	26	tetrads only
V.Q.	—	—	24 to 26	—	pronounced 1(3), maximum 7(2)	—	tetrads occasional triad or pentad
V.T.	very irregular	Nearly all flowers are empty shells, about 13(1) other configurations indeterminate.	17 to 23	nearly every case	—	—	tetrads
V.U.	fairly regular	1(4) 2(3) 18(2) 4(1)	22 to 27	50%	not obvious maximum 6(2)	50	tetrads only
V.V.	regular	2(3) 20(2) 2(1) occasionally 1(4)	25 to 26	occasional	present, maximum 7(2)	48	tetrads only

plant associated with its supposed parents, the plants of this wild population often show such an alteration — the stipules enlarge, their base broadens, and the lateral lobes tend to be carried up the stipule. The different combination of characters occurring among the progeny of three of the plants is direct evidence of the heterozygosity of their parents.

The general cytological behaviour of the plants confirms this assumption of hybridity — they are irregular in varying degrees. At the first meiotic division univalents, bivalents, trivalents, and quadrivalents occur; the bivalents, however, are in the great majority. Conjugation of dissimilar pairs of chromosomes has been noticed. The orientation of the chromosomes on the first division metaphase plate, lagging chromosomes, some of which split, eliminated chromosomes, and finally, the sometimes slight but nevertheless different, numbers of chromosomes at the second division poles, characterise these plants as of hybrid origin.

B. *The Status of the Population*

The chromosome number of *Viola tricolor* is $n = 13$ (CLAUSEN, 1929) and that of *lutea* is $n = 24$ (CLAUSEN, *ibid.*). A cross between these should theoretically yield plants with $2n =$ about 37 and gametes with $n = 18$ or $= 19$. The plants in question, however, have a chromosome number of $2n =$ about 48, with gametes, the majority of which, show $n = 23$ to $n = 26$. Whence arises this?

CLAUSEN (1931) crossed *V. tricolor* with *V. lutea* and found it characterised by great irregularity in the progress of meiosis similar to the irregularities already described but with the occurrence of a large number of univalents which showed a tendency to divide at anaphase. He considered that the splitting of the univalents was responsible for the average increase in the number of chromosomes in the gametes which he found. CLAUSEN (1926) claims to have been the first to demonstrate how an increase of chromosome numbers in interspecific crosses takes place. The plant showing this arose from a cross between *V. tricolor* ($n = 13$) and *V. arvensis* ($n = 17$). In it CLAUSEN found that there were only a few bivalents formed, fewer than 13, but the number varied. The univalents left over divided at both divisions. As a result the second division plates showed a

varying number of chromosomes present, all increased. The subsequent F_2 and F_3 generations of this plant likewise showed an increased number. CLAUSEN considers that this case is an illustration of WINGE's theory of indirect chromosome binding and, further, that it supports the view that new species can arise from crossings between already existing ones.

Subsequent to that paper it has been shown that a class of polyploids — allopolyploids — are hybrids which owe their polyploid nature to the doubling of all their chromosomes. Allopolyploids are probably of common occurrence in Nature.

It may be supposed then that the plants of the wild population arose from the cross *V. tricolor* \times *V. lutea* with subsequent double division of univalents. From this cross 13 bivalents and 11 univalents are possible in the ordinary way. If all the univalents split at both divisions then gametes with many more than the expected number of chromosomes will be produced. CLAUSEN has found from 8–11 univalents in this cross most of which split. The occurrence of 11 univalents makes it seem possible that they are all *lutea* chromosomes which are left over. It is not necessary to postulate this, however, and besides, the occurrence of multivalents renders it possible that some of the chromosomes are conjugating among themselves. The fact that the morphology of the plants shows a strong tendency to resemble *V. lutea* argues that a greater number of *lutea* chromosomes is present.

An increase of chromosome number in the hybrid could also be accounted for if doubling had occurred in the gamete of *V. tricolor* before fertilisation with the gamete of *V. lutea*. On fertilisation a zygote with about $2n = 50$ chromosomes would be produced. The evidence is against this for if it had taken place the subsequent progeny would possess 24 *lutea* and 26 *tricolor* chromosomes. Thus, if the hybrids arose by this method, there would be more *tricolor* than *lutea* chromosomes present in their offspring. The *lutea*-like appearance of the majority of the hybrids would then be more difficult to explain.

The initial hybrid, or hybrids, that led to the production of this wild population cannot be examined. Hence direct evidence as to how the increase of chromosome number was actually brought about is not available. The occurrence of a few univalents, which sometimes

split, in the hybrids with about $2n = 48$ chromosomes, and the occurrence of many univalents, which also may split, in those hybrids with 44 or less somatic chromosomes, may be taken as further evidence indicating that the actual increase in number is brought about by splitting of the unpaired chromosomes of the original crosses. This would give in the wild plants both autosyndesis and allosyndesis: allosyndesis would occur by conjugation of 13 *lutea* with 13 *tricolor* chromosomes and the formation of a small number of multivalents, due, in some cases, to structural chromosomal change. Autosyndesis could occur by conjugation of the split and similar extra *lutea* chromosomes leading to an increase in the number of bivalents present. This is borne out by the fact that, in many cases, there is a large increase in the number of bivalents over the theoretical 13 that could be formed on the *Drosophila* scheme as in V.F., V.H., V.N., V.O., V.U., and V.V.

The cytology then of these plants shows that, due to the increase in chromosome number, new and untried combinations of chromosomes are being carried out by Nature. The tendency of the wild population is to produce plants with the chromosome numbers of $2n = 48$ to $2n = 52$. In the first case the number is that of one of the original parents while in the second it is double the number of the other parent. A secondary balance is thus being established. In some cases a secondary balance has not yet been attained as in the case of V.G., where diads, triads, and pentads, occur with the normal tetrads. In the more extreme cases a plant is so unbalanced in its chromosomal constitution that no reproductive parts or only those of one sex are formed. Examples of this type of abortion are seen in the empty shells of such plants as V.I., V.J., V.K., V.M., V.R., and V.S. In cases where the chromosomal constitution has a new and secondary balance normal perfectly regular tetrads are produced in spite of some irregularities at the meiotic divisions. These irregularities, in fact, lead to stability in some cases, for old chromosomes can then be eliminated. The real significance of the irregular divisions seems to be that they lead to gametes with a varying number of chromosomes. This variation is not really great, chiefly gametes with 23-26 chromosomes are produced.

The chief advantage then of a large wild population of similar hybrids is that opportunity is provided for much inbreeding whereby,

under the local environmental conditions, a process of trial and error among new chromosomal and gene combinations is able to proceed. It would seem that new combinations of chromosomal numbers which are too low tend to be eliminated in the wild population. In these there is probably a lack of balance as indicated by their great irregularity and the unsplit univalents fail to create a new secondary balance which might possibly be successful. Even though such a plant as, for example, V.J. ($2n = 42$), might survive its chances of reproducing itself sexually are much reduced. In such plants, when pollen tetrads are formed, they are misshapen and the plants themselves are shaggy-looking objects compared with the others — their vigour is reduced.

The fact that the plants of the wild population can have a varying chromosome number does not seem to affect the vigour of the population, providing this number is not too low. In fact plants with an oscillating number of chromosomes are known. CLAUSEN (1931b) gives a list of these besides his own case of *Viola canina*. CLAUSEN considers that *canina* maintains itself as a cytologically irregular species with an oscillating number of chromosomes through constant intercrossing between cytologically different types belonging to the species and that it renews the irregularity through occasional outcrossing with *Viola riviniana* and perhaps other species. Furthermore he adds that "*V. canina* may also be a centre for a present-day creation of a number of new species not yet completely differentiated out." This, surely, can also be said for the wild population under discussion.

There remains the interesting case of the plant V.P. This plant was neither pure *V. tricolor* nor pure *V. lutea* but it has the same somatic chromosome number as *V. tricolor*. In its morphology and cytology it proved to be a hybrid. Thus it could have arisen apomictically from a 52 chromosomed hybrid but there is no evidence to show that this has occurred. It could have arisen by intercrossing with another species with a very low chromosome number: this method can be ruled out as no such species grows near the population. Mass elimination of chromosomes in the zygote shortly after fertilisation could account for its origin. Such mass elimination could occur perhaps if the parents were very irregular in their meiotic divisions and had low chromosome numbers — plants like V.J. or V.T. with a

large number of univalents (figure 8). The number of bivalents in V.J. varies from 13–16. If all the univalents were eliminated the result would be plants with from 26–33 chromosomes. Such an explanation as the above has been given by F. NILSSON (1935). One of the plants from an F_1 hybrid of *Festuca arundinacea* \times *F. gigantea*

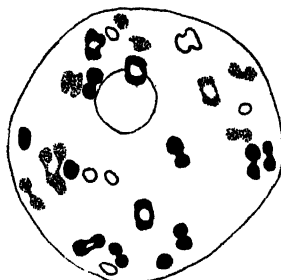


Fig. 8. Diakinesis in V.J. with one trivalent, sixteen bivalents, and ten univalents.

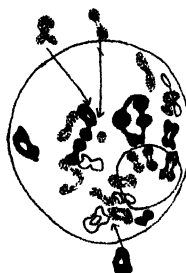


FIG. 9. Diakinesis in V.L. with one ring quadrivalent the components of which are of unequal size.

had 28 instead of 42 chromosomes. Finally the more probable explanation of the occurrence of this hybrid is to assume that it arose by gradual elimination of univalents and lagging chromosomes.

Among the progeny of V.B. 18. were two plants, one with $2n = 27$ and the other with $2n = 30$ chromosomes, also one of the progeny of V.B. had $2n = 24$. Meiosis in these plants is not known. It is to be presumed that they arose in a similar way to V.P. A mass elimination of chromosomes, such as is visualised above, would obviously be of great advantage to a plant like V.J., because then a balanced and stable plant could result with regular reduction divisions. Meiosis, in fact, in V.P. is more regular than in those plants with 48–52 chromosomes. Providing, then, that a plant so produced is strong and vigorous its survival value should be high. Actually V.P. was a healthy looking plant. Thus a new form could be created, probably also with a new phenotypic expression. There is no reason why such a plant should not breed true.

The plants of this wild population of *Violas* are similar in some respects to the pentaploid wheat hybrids of KIHARA (1919, 1921) and

WATKINS (1924, 1930). These writers find that the somatic chromosome number of the F_1 of any cross between species of the Emmer group ($n = 14$) and species of the Vulgare group ($n = 28$) is 35. In subsequent generations, due to random segregation of split univalents, the gametes may have from 14 to 21 chromosomes, and the zygotes 28 to 42. Thus KIHARA was able to divide them into two groups according to whether they had more or less than 35 chromosomes. Those with less showed a tendency to diminish in number and those with more than 35 to increase in number, until, in the end, the plants went back to the condition of the parents. Thus, gradually, intermediate numbers between 28 and 42 were eliminated. In the *Viola* population definite groups cannot be picked out and associated with fertility. Still there is a similarity in that intermediate numbers between 26 and 48 tend to be eliminated, but the number of plants with the chromosome number of the parent with the higher number is much greater than that of the parent with the lower number. It may also be noted that plants with fairly low numbers and very irregular divisions show abnormal flowers indicating that they are, to a great extent, sterile.

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SUMMARY

1. The paper is concerned with the cytology of a wild population of hybrids between *Viola tricolor* L. ($n = 13$) and *Viola lutea* HUDS. ($n = 24$).

2. The morphological characters of the plants of the wild population and the progeny of some of them show that many combinations of parental characters occur, and that mostly the plants, if not intermediate, tend to resemble *Viola lutea* more than *V. tricolor*.

3. The meiotic behaviour of 22 random samples of the wild population, together with that of two plants, is summarised in Table 4.

4. Meiosis in the wild population is further described and it is seen that various stages of irregularity, and varying chromosome numbers are met with. Tetrad formation is seen in some plants with regular or irregular meiosis, while in others with irregular divisions and low chromosome numbers the flowers are often devoid of reproductive parts.

5. The gametic chromosome number varies from 17 to 27 mostly it is from 23 to 26; one plant showed $n = 13$ to 14.

6. Tables 2 and 3 give the variation in somatic chromosome number of the offspring of some of the plants by open pollination in the field.

7. The evidence for hybridity of the plants is discussed.

8. The increase in chromosome number of the hybrids is put down as due to the splitting of univalents, shown by CLAUSEN to occur in similar hybrids.

9. The tendency of the wild population seems to be to produce a majority of plants with chromosome numbers from $2n = 48$ to $2n = 52$. A slight similarity between the wheat hybrids and these plants in this respect is noted.

10. The discussion attempts to show that the interest of a wild population such as this is that it provides a chance for favourable combinations of chromosomes to occur which may lead to the formation of new phenotypes. Variation in chromosome number within the population is an added advantage in this respect.

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A CYTO-GENETICAL STUDY OF THE INTERGENERIC HYBRID "CERAPADUS"

(Michurin Central Laboratory of Genetics of Fruit and Berry Crops,
Michurinsk, U S.S.R.)

by

K. K. YENIKEYEV

(Received for publication June 9th 1937)

"Cerapadus" is a fully fertile hybrid between two genera — the sour cherry (*Pr. Cerasus*) and the bird cherry (*Pr. Padus*). This hybrid was produced by the late I. V. MICHURIN in 1925 by crossing two geographically distant forms, MICHURIN's sour cherry "Ideal" (*Cerasus fruticosa* PALL. \times *C. pennsylvanica* L.) and the Maack bird cherry (*Padus Maackii* RUPR.). MICHURIN made this cross with the aim of creating a hardy and productive variety of cherries, whose fruits would hang in racemose clusters as in the bird cherry. He named the variety he obtained from this cross "C e r a p a d u s". The works of MICHURIN, BURBANK (1914), HANSEN (1908), and others have shown that interspecific hybridization is the best means of originating new varieties of stone-fruits.

Cyto-genetical investigation of such intergeneric hybrids as "Cerapadus" will make it possible to determine how economic characters are inherited in stone-fruits, and will facilitate the work of plant breeders in selecting the initial forms for such distant crosses. In recent years important cytological studies in the genus *Prunus* have been carried out by KOBEL (1927), DARLINGTON (1928–1933), RYBIN (1929–1935), and others. These cytologists arrived at the important conclusion that the basic number of chromosomes in the genus *Prunus* is 8, the various species within this genus forming a polyploid series: 16–24–32–48 (2n).

In DARLINGTON's works the origin of sour cherry-sweet cherry

hybrids has been investigated quite thoroughly and the first attempts were made at a study of the morphology of chromosomes and their conjugation at meiosis.

In a recent paper RYBIN (1936) has given the results of experiments which throw light on the origin of the cultivated plum. From the genetic point of view the genus *Prunus* has not been studied sufficiently. As compared with other species, peaches have been investigated more thoroughly (CRANE, 1929). WELLINGTON (1927) made a study of the inheritance of a number of characters in *Prunus domestica*.

MATERIALS AND METHODS

At our Genetical Laboratory detailed economic and biological descriptions were made of the parental forms from which "Cerapadus" was derived, *i.e.*, the sour cherry "Ideal" and the Maack bird cherry, and of 16 seedlings of the first generation and 97 of the second. This material served as a basis for certain genetic inferences. At the same time, a cytological study was made of the sour cherry "Ideal", the Maack bird cherry, "Cerapadus" No. I (F_1), "Cerapadus" No. II (F_1), and the following F_2 seedlings: "Cerapadus" Nos. 129, 171, 218 and 201. In addition, the complex interspecific hybrid, "Cerapadus" \times "Northern Beauty" No. 23 ("Krasa Syevera"), was also studied. For the cytological studies root-tips and buds were fixed in the field. For the root-tips we used LEVITSKY's fixative (5 parts of 1% chromic acid and 5 parts of 10% formalin); for buds — NAVASHIN's fixative (10 parts of 1% chromic acid, 4 parts of 40% formalin, and 1 part of glacial acetic acid). All the material was embedded in paraffin, and sections were cut on a microtome: root-tips into sections 8 microns thick; buds into sections 15 microns thick. The stain used was HEIDENHAIN's iron-haematoxylin. The drawings in Plates I and II were made with the aid of an Abbé camera lucida; objective 90 \times and ocular 17 \times ; magnification c. 2700.

DESCRIPTION OF THE PARENTAL FORMS

Sour cherry "Ideal". This variety was produced by MICHURIN in 1906 by crossing the steppe cherry (*Cerasus fruticosa* PALL.) with

the Pennsylvania sour cherry (*C. pennsylvanica* L.) "Ideal" is the maternal parent of "Cerapadus". It is a low bush, not over 1,8 m in height. The fruit is light red, globular, medium in size (diam. 14.2 mm weight 2.43 gr), sub-acid, slightly astringent, arranged in umbellate clusters, two or three together on one-year-old growth. (Fig. 1). The yield is abundant. The variety is self-sterile; very hardy.

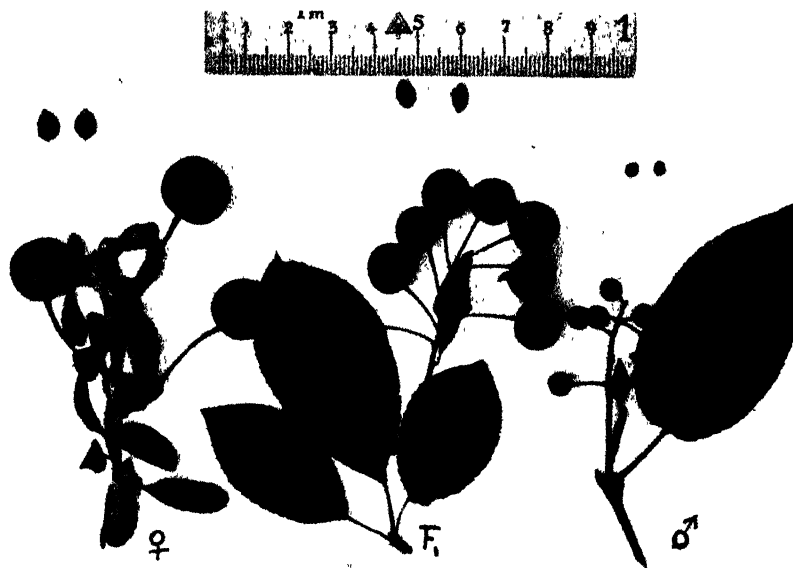


FIG. 1 ♀ Sour Cherry „Ideal”, ♂ Maack Bird cherry (*P. padus Maackii* RUPR), F₁ Cerapadus No. 1

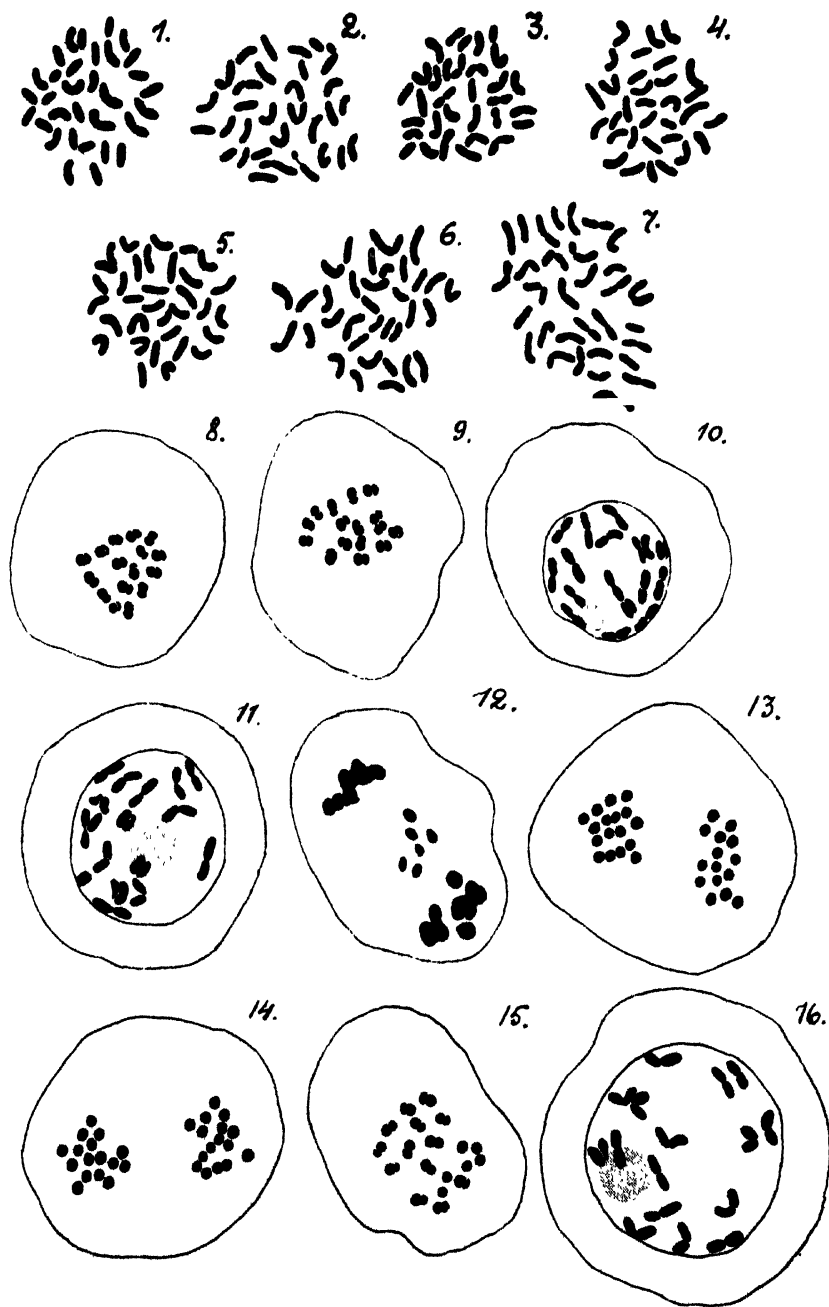
The somatic chromosome number of "Ideal" is 32. (Plate 1, Fig. 1). Its parental forms, *C. fruticosa* and *C. pennsylvanica*, also have 32 as their somatic chromosome number (DARLINGTON 1928, KHARITONOVA, 1934). Meiosis in "Ideal" is, on the whole, regular. Sixteen bivalents are found at diakinesis and at first metaphase (Plate I, Fig. 8). At first anaphase lagging of chromosomes is occasionally observed (one univalent in 9.5 per cent of the cells).

The germinating capacity of the pollen is low -- 1.9 per cent (1935), 2.8 per cent (1933) -- though 80 per cent of the pollen stains with aceto-carmin.

Maack Bird Cherry (*Padus Maackii* RUPR.). This species is the

PLATE I

- 1 Sour Cherry , Ideal", $2n = 32$
- 2 Maack Bird cherry (*Padus Maackii* RUPR), $2n = 32$
- 3 Cerapadus F₁ No 1, $2n = 32$
- 4 Cerapadus F₂ No 129, $2n = 32$
- 5 Cerapadus F₂ No 201, $2n = 33$
- 6 Cerapadus F₂ No 218, $2n = 34$
- 7 Cerapadus \times Northern Beauty $2n = 32$
- 8 Sour cherry , Ideal", metaphase of the 1-st division, 16 bivalents
- 9 Maack Bird cherry (*Padus Maackii*), metaphase of the 1-st division, 16 bivalents
- 10 Cerapadus F₁ No 1, diakinesis 16 bivalents
- 11 Cerapadus F₁ No 1 diakinesis, 14 bivalents + 1 trivalent + 1 univalent
- 12 Cerapadus F₁ No 1, anaphase of the 1-st division chromosomal lagging
- 13 Cerapadus F₁ No 1, metaphase of the 2-d division, 17 + 15 chromosomes
- 14 Cerapadus F₁ No 1, metaphase of the second division 16 + 16 chromosomes
- 15 Cerapadus No 161, metaphase of the 1-st division 13 bivalents + 6 univalents
- 16 Cerapadus F₂ No 129, diakinesis 16 bivalents



pollen parent of "Ceropadus". According to KOMAROV (1903), the Maack bird cherry is an endemic East-Asiatic species with the following area of distribution: the Far Eastern Region (Amur and Ussurian districts), North China (Girin and Mukden Provinces), and Northern Korea. The trees have broad, pyramid-shaped tops, and are of vigorous growth, reaching 11–12 m in height. A characteristic feature of this species is its striking dull yellow, flaky bark, which peels off in broad, transverse strips. The trees are very hardy. The fruits are small (diam. 5 mm., weight 0.4 gr.), black, astringent, bitter, inedible. They hang in racemose clusters, 15–18 per cluster at the beginning of ripening, but later, as the process of ripening progresses, some fruits drop and only 5–7 remain per cluster. (Fig. 1) The yield is low.

Somatic plates showed $2n = 32$ (Plate I, Fig. 2). Meiosis is regular; the chromosomes conjugate, as a rule, normally, 16 bivalents being formed at diakinesis and at first metaphase (Plate I, Fig. 9). At first anaphase chromosome disjunction is usually regular, only in one cell the lagging of one univalent having been noted. The pollen is normal, its germinating capacity being 10–11 per cent; the amount of pollen stained by aceto-carmine is about 88.2 per cent.

SEGREGATION IN THE FIRST AND SECOND GENERATIONS

Table 1 gives some of the most outstanding characters of the parental forms and the inheritance of these characters in the first and second generations. Due to the insufficient number of seedlings (16 in F_1 , 97 in F_2), we cannot draw decisive conclusions. However, we shall try to trace the general trend of segregation in the case of a number of characters. (See table 1 page 193).

Growth. — The mother tree is a dwarf; the pollen parent is of vigorous growth. In the first generation all of the seedlings are tall and vigorous, but in the second generation 19.5 per cent are dwarf forms, 35.1 per cent are of medium height, and 45.4 per cent are tall and vigorous. In general, it may be stated that heterosis was marked in the first generation.

Color of Trunk. — The trunk of the mother tree is dark-brown; that of the pollen parent — yellow. In the first generation the dark color dominates over the yellow color. In the second generation the greater percentage (64%) of the seedlings have dark-brown trunks, only 36 per cent having yellow trunks.

TABLE 1. INHERITANCE OF CHARACTERS IN F₁ AND F₂ OF "CERAPADUS"

Characters	♀	♂	F ₁		F ₂	
			Number of plants	%	Number of plants	%
<i>Growth</i>						
1. vigorous		+	16	100	44	45.4
2. medium					34	35.1
3. dwarf		+			19	19.5
<i>Trunk and branches</i>						
1. yellow		+			35	36
2. dark-brown		+	16	100	62	64
<i>Fruits borne in</i>						
1. racemose clusters . . .		+			23	23.7
2. corymbose clusters . . .			16	100	25	25.7
3. umbellate clusters . . .		+			49	50.6
<i>Fruits</i>						
1. black		+			37	38.1
2. dark red			16	100	42	43.3
3. red		+			18	18.6
<i>Fruits</i>						
1. large		+			37	38.1
2. medium			16	100	21	21.7
3. small		+			39	40.2
<i>Fruits</i>						
1. acid-bitter		+	14	87.5	66	68
2. acid			2	12.5	15	15.5
3. sub-acid		+			16	16.5
<i>Fruits of</i>						
1. poor quality		+	16	100	89	91.8
2. medium quality		+			8	8.2
3. good quality					—	—

Notes. Characters peculiar to the initial forms are marked with +.

Arrangement of Fruit. — The fruits of the mother tree are borne in umbellate clusters, 2 or 3 together; those of the pollen parent — in racemose clusters. The first generation trees are intermediate in this respect, having so-called corymbose clusters. In the second generation segregation is as follows: seedlings with fruits in racemose clusters — 23.7%; in corymbose clusters — 25.7%; and in umbellate clusters — 50.6%.

Color of Fruit. — One of the parents has black fruit; the other — red. In the first generation all seedlings have dark-red fruit, while in the second generation 38.1% have black fruit, 43.3% — dark red fruit, and 18.6% — red fruit.

Size of Fruit. — The mother tree has comparatively large fruit, the pollen parent bears small fruit. The first generation trees are intermediate in this respect, bearing fruit of medium size. In the second generation there appear almost in equal numbers seedlings bearing small fruit (40.2%) and those bearing large fruit (38.1%), and a smaller number of seedlings (21.7%) with fruit of medium size. There is a wide range among the seedlings with respect to size of fruit, from very small to very large.

Flavor and Quality of Fruit. — Though not very good in quality, the fruit of the sour cherry "Ideal" is edible, sub-acid. The fruit of the Maack bird cherry is bitter, astringent, inedible. Most of the F_1 seedlings (87.5%) have bitter, astringent fruit, and the remainder (12.5%) have acid fruit. In the second generation the percentage of seedlings with acid-bitter fruit is 68, with acid fruit — 15.5, with sub-acid fruit — 16.5. It may definitely be stated that in the first and second generations acid flavor dominates over sub-acid. Accordingly, all of the seedlings in the first and a great majority in the second generation have fruit of poor quality, only a very small number of F_2 seedlings bearing fruit medium in quality.

The observed segregation of characters makes it possible to select seedlings of satisfactory productivity with fruits of fair quality borne in racemose clusters.

Time of Flowering. — As regards time of beginning of blossoming, all the seedlings of the first generation are intermediate between the parental forms. In the second generation a few early-blossoming seedlings appear, some seedlings intermediate with respect to date of blossoming, and quite a number blossoming later (1–4 days later

than the parental forms). The last type is of great interest as one insured against late spring frosts. The duration of blossoming in the case of the sour cherry "Ideal" is about 8 days, of the Maack bird cherry — 9 days, of the F_1 seedlings — 7 days, and of the F_2 seedlings — 6.5 days.

Time of Ripening. — The sour cherry "Ideal" begins to fruit on July 18, the Maack bird cherry — on July 5. As regards beginning of ripening, the F_1 hybrids occupy an intermediate position between the two parental forms. In the second generation a considerable variation in this character is observed. In addition to a great majority of intermediate forms, there are some seedlings which ripen very early and some which ripen very late, exceeding in this respect the limits set by the two initial forms. In the second generation two seedlings ripened 4 days earlier than the early-maturing initial form, the Maack bird cherry, and 31 seedlings considerably later than the late maturing initial form, the sour cherry "Ideal" (see Table 2, pag. 196).

As regards length of the fruiting season, in the first and especially in the second generation there appeared forms exceeding the limits set by the initial forms. The fruiting season of four seedlings in F_2 was extremely short, about 4 days, while that of the early maturing initial form, the Maack bird cherry, is not less than 6 days. Likewise, some forms appeared with a very long fruiting season (up to 25 days), exceeding in this respect the late-maturing initial form, the sour cherry "Ideal", whose fruiting season is 14 days.

Weight of fruit. —

Average weight of fruit of the sour cherry "Ideal".	2.43 gr.
" " " " of the Maack bird cherry .	0.4 "
" " " " of F_1 seedlings.	0.61 ± 0.17 "
" " " " of F_2 "	0.72 ± 0.06 "
" " " " of large-fruited F_2 seedlings	2.45 "

With respect to weight of fruit, the limits set by the initial forms are exceeded to only an inconsiderable extent.

Yield. — It is rather difficult to compare the yields of the initial forms and of the hybrids, owing to differences in age and in the conditions under which they are growing. Moreover, it should be noted that one of the parental forms, the sour cherry "Ideal", grows on a stock, while the Maack bird cherry and the hybrids are not

grafted trees. Nevertheless, with respect to yield some interesting points should be noted. In 1934 the yield of the sour cherry "Ideal" was 1917 gr.; that of the Maack bird cherry — 238 gr. The former may be considered a productive form, the latter a shy bearer. Yields of the F_1 seedlings at the age of ten years ranged from 21.7 gr. to 5,853.2 gr. per tree, the average yield (for 15 seedlings) being 1,682.3 gr.

It should be noted that there are very few low-yielding seedlings in the first generation; six seedlings gave abundant yields, greatly

TABLE 2

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	VII	VII	VII	VII	VII	VII	VII	VII	VII	VII	VII	VII	VII	VII
F_1						1		2		5		1		6
F_2	2					3	2	7	3	10	1	3	4	3

	F_1		F_2	
	Number	%	Number	%
1. Number of hybrids ripening their fruit before the beginning of ripening of the Maack bird cherry	—	—	2	2.4
2. Number of hybrids intermediate as regards time of ripening . .	15	100	49	59.8
3. Number of hybrids ripening their fruit after the sour cherry "Ideal"	—	—	31	37.8
Total	15	100	82	100

exceeding that of the more productive parental form "Ideal". The 97 seven-year-old F_2 seedlings may be grouped, according to yield, in the following manner:

Variation in yield (in grams per tree)	Number of seedlings
0.7-102.0	83
102.1-203.4	8
203.5-304.8	3
304.9-406.2	—
406.3-507.6	—
507.7-609.0	1
609.1-710.4	2

16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	1
VII	VII	VII	VII	VII	VII	VII	VII	VII	VII	VII	VII	VII	VII	VII	VIII
3	5	4	1	12			4	4	3	3	3				1

The average yield of these F_2 seedlings (second year of fruiting) was about 60 gr.

From this data it is evident that there is a great variation in yield in the second generation. Rogues appear, yielding little or practically no fruit (10-20 grams per tree). The cause of the low productivity of these seedlings lies in the irregularities at meiosis connected with intergeneric hybridization. At the same time, some seedlings, already in the second year of fruiting, yielded better than others (600-700 gr. per tree), and, undoubtedly, in subsequent years they will exceed in yield the more productive initial form, "Ideal".

Since the two species crossed were both hardy, exceptionally hardy F_1 and F_2 progeny were obtained. The fruit of all of the "Cera-padus" seedlings is characterized by resistance to fungus diseases, particularly fruit rot (*Monilia cinerea*), while as much as 25 per cent of the fruit of the sour cherry "Ideal" is infected. The principal defect of most of the "Cera-padus" seedlings is the poor quality of the fruit, only occasional specimens occurring with edible fruit of fair quality. Nevertheless, "Cera-padus", owing to its great hardiness, fair productivity, and immunity to fungus diseases, is of much interest as initial material for further plant-breeding work. Moreover, "Cera-

padus" may be used as a sturdy stock for propagating cultivated varieties of sour cherries and as a unique, rapidly growing ornamental tree.

CHARACTER CORRELATIONS

An analysis of the 97 F_2 seedlings revealed the following correlations:

1. Size of fruits and their arrangement	0.64 \pm 0.08
2. Size of leaves and weight of fruit	0.43 \pm 0.08
3. " " " " arrangement of fruit	0.45 \pm 0.08
4. Flavor and size of fruit	0.30 \pm 0.09
5. Color of bark and arrangement of fruit	0.30 \pm 0.09
6. Color of bark and size of fruit	0.24 \pm 0.09

A definite correlation is observed between the size of the fruits and their arrangement (racemose or umbellate clusters, etc.). As a rule, when the fruits are small, they are borne in racemose clusters, while large fruits are borne singly. Evidently, it is necessary to have a great number of seedlings, in order to get the desired combination of large fruits borne in racemose clusters. A few such seedlings occurred in the second generation. There is some correlation between the size of leaves and both the weight and arrangement of fruit. No correlation was found between flavor and size of fruit or between color of bark and size or arrangement of fruit.

DESCRIPTION OF THE PRINCIPAL TYPES OF "CERAPADUS"

The chief types of seedlings obtained by crossing sour cherry with bird cherry are:

Type 1 — intermediate between the initial forms. Such seedlings occur mainly in F_1 , occasionally in F_2 .

Type 2 — resembling the mother plant, the sour cherry "Ideal".

Type 3 — resembling the pollen parent, the Maack bird cherry.

Type 4 — low-yielding forms.

The second, third and fourth types occur in the second generation.

We shall now give a brief description of these seedling types and the results of our cytological analyses of them.

Type 1 — F₁ "Cerapadus" No. 1.

Tree with a broad, round top, of vigorous growth, at the age of 10 years reaching 4.5 m. in height. Fruit globular, dark red, small (diam. 11–12 mm., weight 0.6 gr.), subacid, highly bitter, inedible, borne in corymbose clusters, 5–8 in each (Fig. 1, p. 189). Yield very high — 5–6 kg. per tree. Tree hardy, very resistant to insect pests and fungus diseases, self-sterile. Pollen germination good, 16.5%, though the percentage of pollen stained with aceto-carmin is not large — 51.5.

The somatic chromosome number was found to be 32 (Plate I, Fig. 3), *i.e.*, the same as that of the parental forms. Though the hybrid is distinguished by its high productivity, considerable irregularities, as compared with the initial forms, are observed at meiosis. At diakinesis there are found not only cells with regular conjugation of chromosomes, 16 bivalents (Plate I, Fig. 10), but also some cells with 14 bivalents + 1 trivalent + 1 univalent (Plate I, Fig. 11). The separation of the chromosomes to the opposite poles at first anaphase is often irregular. Lagging of chromosomes at first anaphase is of common occurrence (in 56.6% of the cells), frequently as many as six univalents lagging in one cell (Plate I, Fig. 12), but only in one case was a lagging bivalent observed. At metaphase of the second division chromosome distribution is not always regular with 16 chromosomes passing to each pole (Plate I, Fig. 14); sometimes chromosome distributions of 17 and 15 (Plate I, Fig. 13) or 18 and 14 occur.

Such irregularities at meiosis in the F₁ hybrids "Cerapadus" No. 1 are the reason for the appearance of aneuploid forms in the second generation. In general, the most characteristic type of irregularity at meiosis in these F₁ hybrids is the formation of univalents. Notwithstanding the lagging of chromosomes at first anaphase, the number of chromosomes in the two plates at second metaphase always totalled 32. No isolated chromosomes lying in the plasma and not included in the groups at the poles were observed. Tetrads are of normal appearance.

Type 2 — F₂ Seedling No. 161

Medium-sized shrub with a broadly rounded top. Fruit round, dark red, comparatively large, about the size of the sour cherry "Ideal" (diam. 13.7 mm., weight 1.5 gr.), acid with a hardly noticeable bitter taste, quality poor (Fig. 2). A shy bearer; self-sterile; hardy.

The somatic chromosome number is 32. Meiosis is irregular. At first metaphase 13 bivalents and 6 univalents are observed (Plate I, Fig. 15), and at first anaphase lagging of chromosomes. Pollen germinates poorly (0.4%) and stains but slightly with aceto-carmin (36%). In general habit of growth this seedling is quite similar to the sour cherry. The decrease in productivity is evidently due to the irregularities at meiosis and the consequent abnormal formation of pollen.



FIG. 2. Cerapadus F₂ No. 161.

Type 3 — F₂ seedling No. 129

Tree vigorous, with a broad pyramid-shaped top. Fruit globular, small (diam. 7 mm., weight 0.2 gr.), black, acid-bitter, inedible (Fig. 3), borne in racemose clusters, 4 to 7 in each. Yield medium. The seedling is self-sterile; hardy.

The somatic chromosome number is 32 (Plate I, Fig. 4). Meiosis is normal; at diakinesis 16 bivalents are formed (Plate I, Fig. 16). At

first anaphase there was observed a single instance of the lagging of one univalent, which also has been found to occur in the case of the Maack bird cherry. Pollen germination is fair — 7.8%. In general habit this seedling very closely resembles the Maack bird cherry.

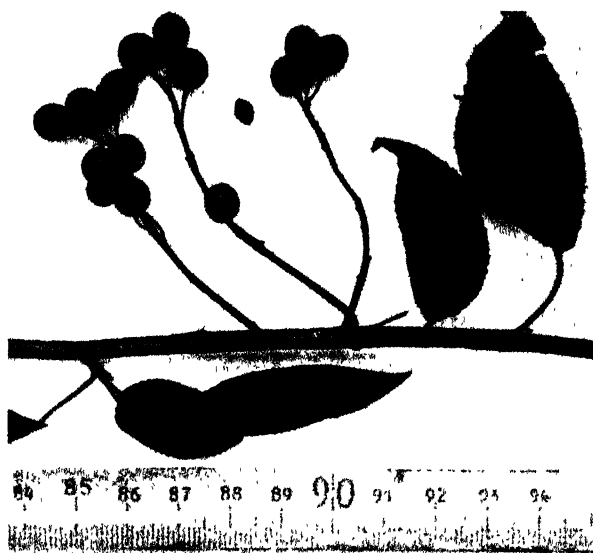


FIG. 3. Cerapadus F₂ No. 129.

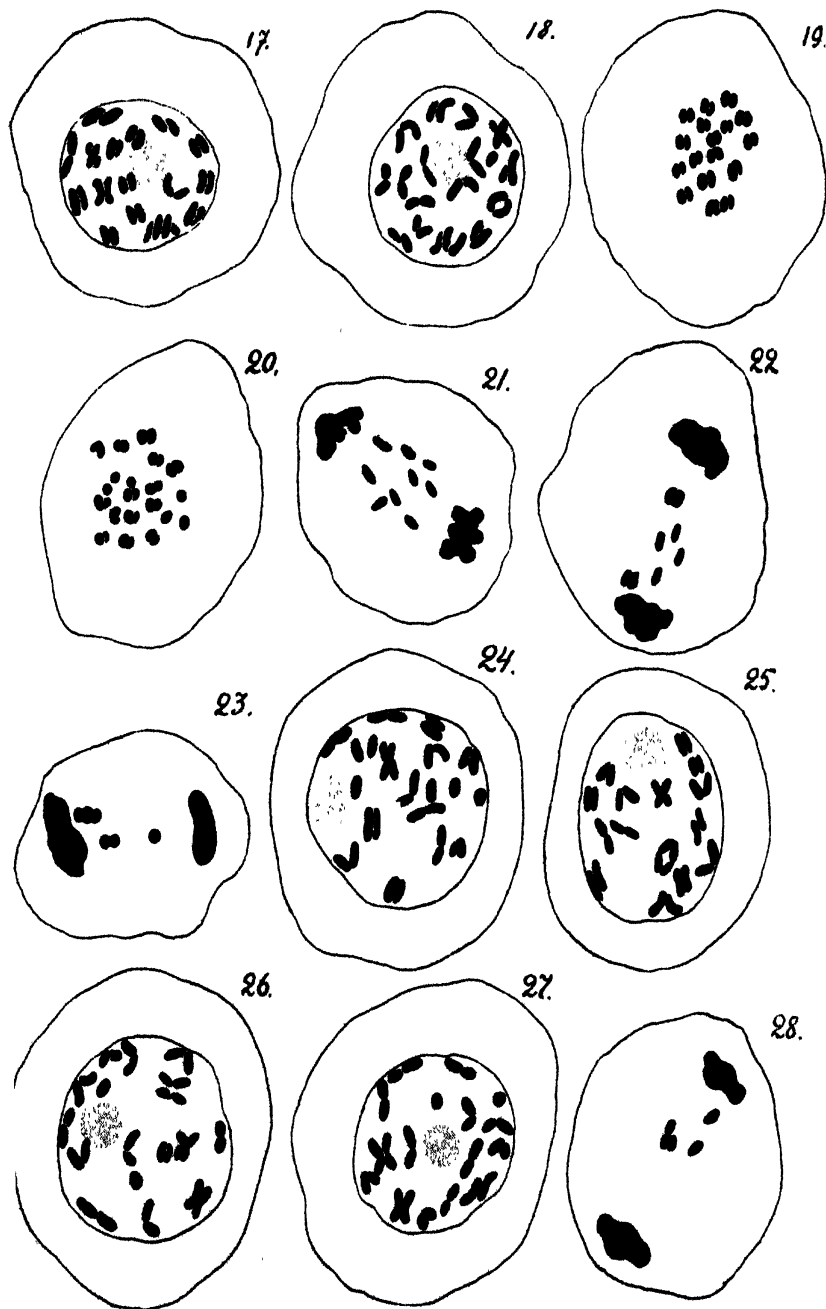
Type 4 — F₂ seedling No. 218

Low tree with a broadly rounded top. Fruit large (diam. 14 mm., weight 1.5 gr.), sub-acid, bitter (Fig. 4), borne in loose, corymbose clusters, 1–2 in each. The seedling is low-yielding, self-sterile. Most of the flowers in a raceme do not set fruit, and, consequently, at the time of ripening single fruits occur.

The somatic chromosome number was found to be 34 (Plate I, Fig. 6). Great irregularities are observed at meiosis. At diakinesis and first metaphase univalents, trivalents and quadrivalents occur. At diakinesis the following types of chromosome configuration have been found: 15 univalents + 1 tetravalent (Plate II, Fig. 17), 15 bivalents + 1 univalent + 1 trivalent (Plate II, Fig. 18); at first metaphase the following: 17 bivalents (Plate II, Fig. 19), 13 bivalents + 1 trivalent + 5 univalents (Plate II, Fig. 20). At first ana-

PLATE II

17. Cerapadus F₂ No. 218, diakinesis, 15 univalents + 1 tetravalent.
18. Cerapadus F₂ No. 218, diakinesis, 15 bivalents + 1 univalent + 1 trivalent.
19. Cerapadus F₂ No. 218, metaphase of the 1-st division, 17 bivalents.
20. Cerapadus F₂ No. 218, metaphase of the 1-st division, 13 bivalents + 1 trivalent + 5 univalents.
21. Cerapadus F₂ No. 218 chromosome lagging at anaphase of the 1-st division.
22. Cerapadus F₂ No. 218 anaphase of the 1-st division, chromosome lagging.
23. Cerapadus F₂ No. 218 anaphase of the 1-st division, chromosome lagging.
24. Sour cherry „Northern Beauty”, diakinesis, 13 bivalents + 6 univalents.
25. Sour cherry „Northern Beauty” diakinesis, 14 bivalents + 1 tetravalent.
26. Cerapadus × „Northern Beauty”, diakinesis 15 bivalents + 2 univalents.
27. Cerapadus × „Northern Beauty”, diakinesis 13 bivalents + 1 tetravalent + 2 univalents.
28. Cerapadus × Northern Beauty, chromosome lagging at anaphase of the 1-st division.



phase in 76.9 per cent of the cells there are a considerable number of lagging chromosomes, sometimes as many as 9 in one cell (Plate II, Fig. 21). The laggards are most frequently univalents, less often trivalents and bivalents (Plate II, Figs. 22, 23). The pollen scarcely germinates at all and does not stain with aceto-carmin. This hybrid is an aneuploid form. The two extra chromosomes in this seedling give rise to the formation of quadrivalents and trivalents not observed in

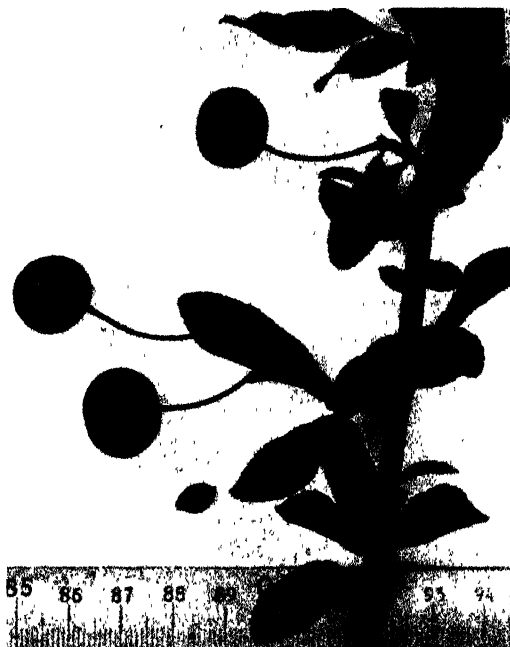


FIG. 4. *Cerapadus* F₂ No. 218.

the 32-chromosome F₂ forms. Its pollen sterility and its very poor productivity (it may be considered as practically non-productive) seem, to a certain extent, to be connected with its aneuploidy. In general habit the seedling differs greatly from the initial forms. The F₂ seedlings Nos. 171 and 201 (Fig. 5) should also be considered aneuploid forms, since they have 33 as their somatic chromosome number. (Plate I, Fig. 5).

THE HYBRID "CERAPADUS" \times "NORTHERN BEAUTY"

[(*Cerasus fruticosa* PALL. \times *Cerasus pennsylvanica* L. \times *Padus Maackii* RUPR.) \times (*Cerasus vulgaris* MILL. \times *Cerasus avium* L.)]

The cross between "Cerapadus" and the sour cherry "Northern



FIG 5. Cerapadus F₂ No 201.

Beauty" ("Krasa Syevera") was made with the aim of improving the quality of fruit of "Cerapadus". The hybrid obtained is of great theoretical interest as a complex, interspecific hybrid, in the formation of which five species, participated: *Cerasus fruticosa*, *Cerasus pennsylvanica*, *Padus Maackii*, *Cerasus vulgaris* and *Cerasus avium*. Before proceeding to an analysis of the hybrid itself, we shall give a brief description of the pollen parent. For the description of the mother plant, "Cerapadus No. 1", see page 198.

"Northern Beauty" (*Cerasus vulgaris* MILL. \times *Cerasus avium* L.).

— "Northern Beauty" was produced by MICHURIN in 1888 from a cross between "Belle", a Vladimirsky sour cherry, and "Winkler White", a sweet cherry. The tree is of rather vigorous growth, 3.2 m. in height, with a broadly rounded top; medium hardy. Fruit large (diam. 25 mm., weight 5.5 gr.), light pink, sweet with a slight acidity, of excellent flavor. Under conditions at Michurinsk the yield is average. The variety is self-sterile.

The haploid chromosome number, according to our data, is 16 ($2n = 32$). According to DARLINGTON's theory (1928), such fertile sour cherry-sweet cherry hybrids originated from the union of a normal sour cherry gamete with an unreduced sweet cherry gamete. In "Northern Beauty" at diakinesis 13 bivalents and 6 univalents (Plate II, Fig. 24) are usually found, but, as an exception, in one case 14 bivalents and 1 tetravalent were found (Plate II, Fig. 25). At first anaphase lagging of chromosomes occurs to a slight extent — from one to four univalents in 13.9 per cent of the cells. Pollen germination is low (0.5% in 1935); 70.3% of the pollen grains stain with aceto-carmin. The fusion of a normal sour cherry gamete with an unreduced sweet cherry gamete in such hybrids as the "Northern Beauty" results in their fertility. However, the incomplete homology of the chromosomes leads to the formation of univalents and partially abortive pollen, which, apparently, is the cause of the low productivity of the "Northern Beauty".

F_1 "CERAPADUS" \times NORTHERN BEAUTY" NO. 23

The tree at 7 years of age is 3.1 m. high with a broadly rounded top. It is very hardy. Fruit single or in twos on one-year old shoots, roundish-flattened, small (about the size of a small sour cherry; diam. 13.2 mm., weight 1.3 gr.), dark red, sub-acid and bitter, inedible (Fig. 6). Fruit resistant to fungus diseases. Yield low. The hybrid is self-sterile. As regards many economically important characters and general habit of growth, the characters of the mother plant, "Cerapadus No. 1", are dominant. Unfortunately, in the first generation no considerable improvement in quality and increase in size of fruit have as yet been attained. The second generation is of much interest, for in such a complex, interspecific hybrid wide segregation is to be expected.

The somatic chromosome number is 32 (Plate I, Fig. 7). Many irregularities are observed at meiosis. At diakinesis the cells form such configurations: 15 bivalents + 2 univalents (Plate II, Fig. 26), 13 bivalents + 1 tetravalent + 2 univalents (Plate II, Fig. 27). At anaphase of the first division from 1 to 8 laggards are observed in 79 per cent of the cells. The following types of chromosome lagging have been noted: only univalents; univalents and bivalents; univalents and trivalents (Plate II, Fig. 28).

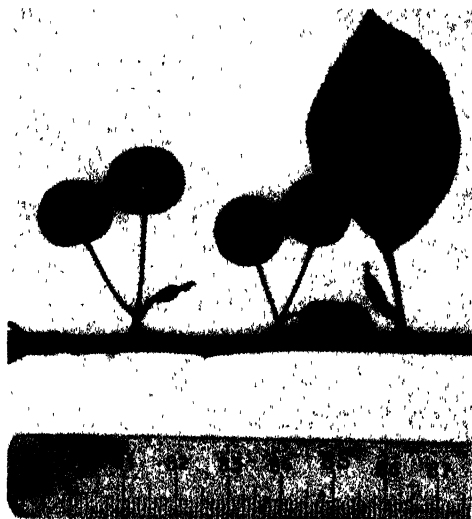


FIG. 6 *Cerapadus* × Northern Beauty No. 23.

The pollen is highly abortive; pollen germination — 1.4%; percentage of pollen grains staining with aceto-carmin — 20.5. The low productivity of this interspecific hybrid is chiefly due, apparently, to the irregularities at meiosis, which lead to the formation of abortive pollen.

MEIOTIC ANOMALIES

According to the anomalies occurring at meiosis of pollen mother-cells, "Cerapadus" seedlings may be classified into several groups:

Group I — The F_1 seedlings of this group (e.g., "Cerapadus" No. 1) have 32 as their diploid chromosome number, and are characterized

by the formation of a considerable number of univalents. At anaphase of the first division as many as 56 per cent of the cells have chromosome laggards. The irregular separation of these univalents at first anaphase gives at second metaphase plates with such numbers: 17 and 15, 18 and 14. The formation of univalents may be explained by the non-homology of some of the chromosomes of the two parents. Disturbances at meiosis in seedlings of the first generation, resulting in the formation of gametes with 17 and 15 chromosomes instead of the normal 16 chromosomes, lead to the occurrence of aneuploid forms in the second generation. Thus, by crossing two geographically distant forms, hybrids are obtained which already in F_1 show considerable irregularities at meiosis, though their fertility is not affected. The pollen of such seedlings germinates well and stains satisfactorily with aceto-carmin. Similar seedlings occur also in F_2 .

Group II — The seedlings of this group occur exclusively in the second generation. Their somatic chromosome number is 33–34 (e.g., seedlings Nos. 218, 171). At meiosis cells with chromosome laggards occur in much greater number (up to 86%). These laggards are not only univalents (though univalents constitute the majority), but in some cases include a trivalent or a quadrivalent. The presence of one or two extra chromosomes in all probability is the cause of great irregularities at meiosis. Most of these seedlings are shy bearers. Their pollen germinates and stains with aceto-carmin poorly.

Group III — The seedlings of the third group are fairly close to the initial forms; their diploid chromosome number is 32. Regular conjugation of chromosomes is a typical feature. Occasional lagging of univalents at anaphase of the first division may be considered a rare exception. The pollen germinates and stains with aceto-carmin well.

No isolated chromosomes in the plasma outside the spindles were observed in any of the investigated seedlings. Tetrad formation is normal; pollen degeneration, in all probability, commences later.

(See Table 3 page 210).

★

DISCUSSION

The cross between the sour cherry "Ideal" and the Maack bird cherry, from which MICHURIN produced the hybrid "Ceropadus".

must be classed as a difficult cross. Prof. G. D. KARPECHENKO (1935) writes that in such distant but "congruent" crosses (as distinguished from crosses between closely related forms) the parental forms differ from one another by a very large number of genes, but they "possess 'corresponding' chromosomes, which may be combined in the hybrids without any decrease in viability or fertility, at least in most cases" (p.293).

Most of the outstanding varieties of fruits created by MICHURIN have in their pedigrees hybridized species from geographically distant regions. Excellent apple varieties for the central zone were produced by MICHURIN by crossing southern cultivated varieties (*Malus domestica*) with the Chinese apple (*M. prunifolia*). He obtained hardy table varieties of pears by crossing the wild Ussurian pear (*Pyrus ussuriensis*) with French cultivated varieties (*P. communis*).

The American plant breeder, N. E. HANSEN, by crossing the Chinese plum (*Prunus triflora*) with American species of plums and sour cherries (*Pr. americana*, *Pr. Besseyi*) has brought out a number of excellent, hardy varieties of plums for northern districts of America. Thus, by means of distant interspecific crosses, especially those involving species from geographically distant regions, dozens of economically valuable varieties have been created.

Among the "Cerapadus" hybrids there are a great diversity of forms as regards habit and vigor of growth, productivity, size and flavor of fruit, time of ripening, etc. In the first generation heterosis or hybrid vigor is observed, being evidenced by the vigorous development of all seedlings, JONES (1917) explains this phenomenon by the accumulative effect of various dominant genes. In the second generation there is considerable variation in habit — from dwarf forms of the type of the steppe cherry to vigorous trees of the type of the Japanese bird cherry. As regards a number of characters, the seedlings exceed the limits set by the parental forms. By crossing two forms, one early-ripening and the other late-ripening, very early-ripening, intermediate, and very late-ripening seedlings were obtained. As regards yield, the same is observed. Seedlings occur with yields several times as great as those of the parental forms, and at the same time there are seedlings, though not so many, of low productivity. New characters appear in the hybrids. Their fruit, as distinguished from that of the two parental forms, is exceptionally

immune to fungus diseases, in particular, to *Monilia*. As to size of fruit, there are two seedlings having somewhat larger fruit than the sour cherry "Ideal".

TABLE 3. CHROMOSOME LAGGING AT FIRST

Serial No.	Name of plant	Chromosome number (haploid)	No. of investigated cells			No. of cells with chromosome		
			Total	With chromosome laggards	% with laggards	Univalents	Bivalents	Trivalents
1	Sour cherry "Ideal"	16	21	2	9.5	2	—	—
2	Maack bird cherry	16	26	1	3.8	1	—	—
3	"Ceropadus" F ₁ No. 1	16	30	17	56.6	14	1	—
4	"Ceropadus" F ₂ No. 218	34/2	26	20	76.9	16	1	—
5	"Ceropadus" F ₂ No. 197	16	15	13	86.6	11	—	1
6	"Ceropadus" F ₂ No. 129	16	14	1	7.1	1	—	—
7	"Northern Beauty" (Krasa Syevera)".	16	43	6	13.9	6	—	—
8	"Ceropadus" × "Northern Beautv" No. 23	16	34	27	79.4	24	—	—

The inheritance of characters in "Ceropadus" does not fall within the framework of ordinary Mendelian segregation. The exceptional wealth of forms observed among "Ceropadus" seedlings of the second generation must be regarded as a result of hybridizing two genera from geographically distant regions. In his works MICHURIN elucidated the dominance of characters in such crosses. He regarded the hereditary basis as a substance in process of development and dominance of characters as the development only of those potentialities of zygotes favored by the given conditions. MICHURIN (1933) writes: "The farther the plants being crossed are separated from one another by their habitat and environmental conditions, the more easily the hybrid seedlings adapt themselves to environmental conditions in the new locality. I explain this by the fact that in such a case the characters of the parental plants or of their close relatives

transmitted to the hybrids, not finding the customary environmental conditions of their original habitat, will not be able to dominate too much in the development of the hybrid organism, which is of

ANAPHASE IN "CERAPADUS" AND ITS PARENTS

various types of laggards			No. of cells with given numbers of chromosome laggards								
Univalents + bivalents	Univalents + trivalents	Univalents + bivalents + trivalents	1	2	3	4	5	6	7	8	9
—	—	—	2	—	—	—	—	—	—	—	—
—	—	—	1	—	—	—	—	—	—	—	—
2	—	—	5	2	2	3	3	2	—	—	—
2	—	1	5	8	2	1	—	2	—	1	1
1	—	—	7	5	1	—	—	—	—	—	—
—	—	—	1	—	—	—	—	—	—	—	—
—	—	—	3	2	—	1	—	—	—	—	—
2	1	—	8	8	4	1	4	1	1	—	—

immense significance in practical breeding work" (p. 28). On the basis of this hypothesis MICHURIN advanced distant hybridization as one of the most important principles in his work. He consciously selected parental pairs widely separated from each other and from the locality in which it was planned to grow the new variety, basing his selection on a thorough study of differences in environmental conditions.

In his outstanding investigations based on the theory of phasic development LYSENKO (1935) experimentally corroborated MICHURIN's views on the dominance of characters. As a result of numerous crosses of wheat, carried out on the basis of a phasic analysis of the initial forms and of the hybrids, LYSENKO arrived at the conclusion that: "dominance is the development of this or that allelomorphic side of the hereditary basis (of the heterozygote) whose requirements

are met by the given environmental conditions, the other allelomorphic side of the heterozygote being unable to develop, due to the necessary conditions being lacking or to their being less favorable for its development" (LYSENKO and PREZENT, 1935, p. 35).

Among "Ceropadus" seedlings we noted the following new qualities: higher productivity, earlier and later ripening as compared with the initial forms, resistance to fungus diseases, etc. The appearance of forms with new qualities from crossing two genera from geographically distant regions may be explained by the fact that the new environmental conditions under which the seedlings are growing differ greatly from those under which the parental forms grew. Cytological studies of "Ceropadus" revealed various irregularities at meiosis, in particular, incomplete chromosome conjugation (at diakinesis and first metaphase), formation of univalents, trivalents and quadrivalents, and lagging of chromosomes at first anaphase.

A. A. SAPEGIN (1928), in cytologically analyzing intervarietal wheat hybrids, found that in some of them there occurred considerable disturbances at meiosis, the more distant from each other the geographical regions of origin of the parent plants, the greater and more frequent being these disturbances. A. A. SAPEGIN (1928) and L. A. SAPEGIN (1933) expressed the opinion that in this case, apparently an accumulation of differences in genes takes place affecting meiosis. Irregularities at meiosis in "Ceropadus" arise from the intergeneric hybridization of two geographically distant forms, differing in a great number of genes. Evidently, in these two genera not all the chromosomes — due to their structure, genic constitution, and arrangement — can conjugate normally, which is evidenced by the appearance of aneuploid forms in the second generation.

SUMMARY

1. The production of a fully fertile intergeneric hybrid between the sour cherry and the bird cherry is of great theoretical and practical importance. A study of 16 F_1 and 97 F_2 seedlings showed an extensive segregation of economic and biological characters and an exceptional diversity of forms. As regards a number of characters, such as time of flowering and fruiting, productivity, etc., the limits set by the parental forms were exceeded.

Cytological studies of the initial forms — the sour cherry "Ideal" and the Maack bird cherry — showed that their chromosome number is 32 ($2n$) and that regular chromosome conjugation at meiosis is typical for them. In "Ceropadus" No. 1 (F_1) the somatic chromosome number is also 32, but, in addition to regular chromosome conjugation, at diakinesis and first metaphase univalents are formed, more rarely trivalents, which lag to a great extent at first anaphase. At second metaphase the plates do not always contain 16 chromosomes each, but sometimes 17 and 15.

In the second generation there occur normal seedlings with 32 as their somatic chromosome number and aneuploid forms; some of the latter have 33 and others 34 as their somatic chromosome number. The appearance of aneuploid forms in the second generation may be explained by the irregularities at meiosis in F_1 , resulting in the formation of 17- and 15-chromosome gametes instead of 16-chromosome ones. In the aneuploid forms irregular chromosome conjugation and the formation of univalents and trivalents are typical. One of the 34-chromosome aneuploids is a tetrasomic; at diakinesis a quadrivalent is formed. Aneuploid seedlings have low productivity. Regular conjugation of chromosomes and normal productivity are characteristic of the 32-chromosome F_2 seedlings. The extensive segregation of economic and biological characters and the irregularities at meiosis in "Ceropadus" may be regarded as the result of hybridizing two geographically distant genera and of raising the hybrids under new conditions differing from those of the initial forms, which undoubtedly has an influence on the appearance of some characters and the suppression of others. Certain "Ceropadus" varieties are highly productive, quite hardy, and immune to fungus diseases, and may be used as initial material in raising sour cherry-

bird cherry hybrids with sweet fruit. The chief drawback of the "Ceropadus" hybrids is the low quality of their fruit.

2. A complex, interspecific hybrid — "Ceropadus" \times "Northern Beauty" ("Krasa Syevera") — has been investigated: [(*Cerasus fruticosa* \times *Cerasus pennsylvanica* \times *Padus Maackii*) \times (*Cerasus vulgaris* \times *Cerasus avium*)]. Its chromosome number is 32 (2n). Irregular chromosome conjugation, the formation of univalents, trivalents, and quadrivalents are typical of this hybrid. Pollen is abortive; productivity poor. Fruit small, inedible. In the F₁ "Ceropadus" characters dominate to a great extent.

In conclusion, the writer wishes to express his sincere gratitude to S. Í. ISAYEV and D. F. PETROV for their great help in carrying out this work.

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BERICHTIGUNGEN

Zu Genetica XX, afl. 1 en 2, Art. Sanders: Die Heredität des Albinismus

Seite 108, Fall 107: Fig. 30 und 39 *muss sein*: Fig. 29 und 30

„ 116, Zeile 5 von oben: Fig. 29 *muss sein*: Fig. 39

Die Figur 39 ist versehentlich im Artikel ausgelassen. Dieselbe wird umstehend abgedruckt.

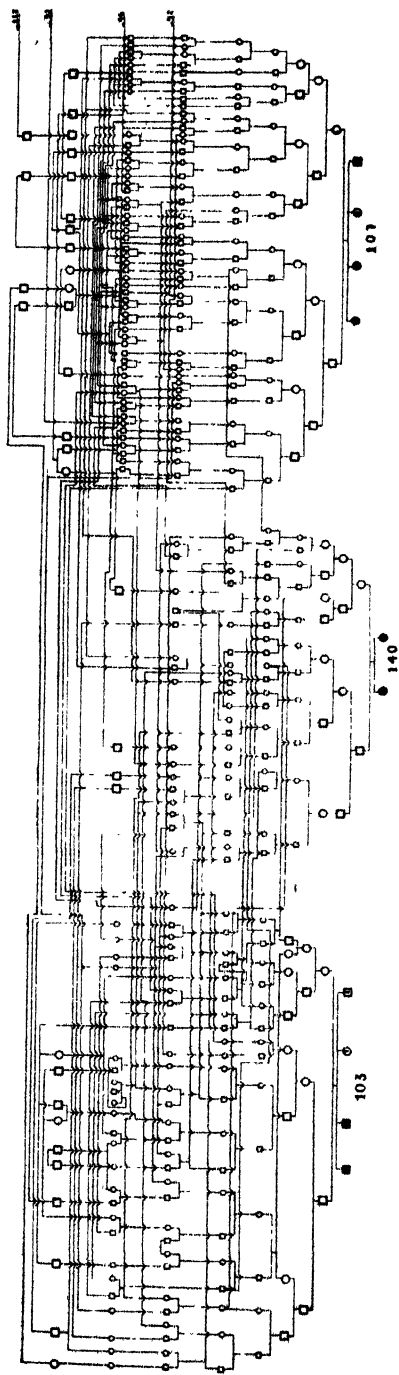


Fig. 39

SOME ANIMAL SPECIES-CROSSINGS IN NATURE WITH ANALYSES OF SIMILAR ONES IN CULTURES, TOGETHER WITH SOME FUNDAMENTAL QUESTIONS. DISCUSSED

by

G. FALKENSTRÖM

Stockholm

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With 3 plates

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A. GENERAL ORIENTATION

Since the last turn of the century the science of heredity — Genetics — has advanced to a predominant position within Biology,

before all after its engaging in the study of the cell. The knowledge of Life on our earth has gained much thereby which must be gratefully admitted. Now it would be desirable if the geneticists, especially the spokesmen in texts and handbooks, did not stop at the details of the laboratories, but searched for corresponding things in living nature. It is of course the latter which is the main point and in the long run a problem to be solved as much as lies in human power. Nevertheless one can raise objections, in good reason I think, against some of the earlier genetic authors, since they waste much of the intellectual value procured for Biology by DARWIN and his followers, seemingly in order to enforce attention to their divergent doctrines. Judging from the retired position which Biology holds in public debates and in our schools at the present time, we have nothing more to lose by the isolation and the one-sided specialization of those who have to plead henceforth for heredity.

In another place (FALKENSTRÖM 1935 ¹⁾) I have commented on the looseness of the conceptions which Genetics has introduced as regards the basis of the scientific Systematology of animals and plants and by that also to the theory of evolution. Because of deficient experience in systematical working, many scientists especially in Germany disclaim the objective realness of the systematical units — the species and all the other ones of higher or inferior rank — and declare them to be human inventions — offsprings of the brain. In Nature, they say, only individuals are to be found. This is true, but only partially, since all individuals are not alike and not of the same kind. The form groups more or less separated from each other by means of the systematic characters which the individuals bear. These characters are measurable as to number, extent and volume and can be drawn, photographed etc. Because of that, they are as real as the individuals which bear them. On these characters alone the systematical groups or units are based, on account of which these are consequently also real things. If any group is erroneously understood and detailed by its author, this fact does not deprive the group and still less all the other adequately detailed groups of their realness. More and better material (i.e. specimens) of

¹⁾ *vide* the note under References.

the group in question, a better mode of investigation, more ability or experience in the investigator, are able to give to the group its due. It is of the greatest importance for man to understand fully and clearly that the systematical groups are real things and by means of their specific characters proved in an objective way as real quantities. Only thus will man be able to see and understand the regularity and lawfulness in the apparent chaos among the living subjects of Nature. LINNÉ found that everyone of most groups of organisms practically showed within itself uniformity in the construction of the individual bodies and that its individuals with respect to some characters were divergent from all other individuals. He named these groups species. He found also that the species were more or less different from other species with regard to the individual characters. Some species which seemed to him to be closely allied and only different in a few characters, he brought together as a higher unit — the genus. Some of the genera which seemed to be more closely allied to each other than to other genera, likewise with respect only to the individual characters, he united to a still higher unit, the family -- and so on. In this way LINNÉ and his followers have arranged their systems of plants and animals by means of the inherent individual characters.

It is evident that LINNÉ and all other systematists after him have subordinated the systematical groups and their individuals under a continued series of higher units. This arrangement is not a guess, a work of chance, but it is enforced by the material itself. LINNÉ, considering the organisms as created in the beginning such as he saw them, i.e. unchangeable, classified the individuals and their groups according to the greater or less degree of conformity in their characters. All other systematists have followed him. But whence does this conformity, visible in the individuals, really come? It is answered, in the main at least, by LAMARCK's and DARWIN's doctrine of descent. Conformity of characters in the living things of the present day is possible only under the condition that it has descended in an unbroken series of generations from

the same ancestral origin. This opinion was expressed by DARWIN in his "Origin of species", and he added also that the systematics' "natural systems" if correctly detailed, would enable us to see the "genealogical" relationship between the members of the group or groups. The individual characters and the groups of individuals carrying the different characters therefore constitute highly important proofs of the truth of the doctrine of descent. The simple fact that one and the same individual of most animals shows in its bodily structure characters by means of which one can read off the complete series of the subordinate groups or units of the Systematics from the base to the top is irrefutable evidence of common descent. If the organisms had had their origin quite independent of each other, the living world would consist only of groups of individuals corresponding to our lowest unit, the species, but without higher units and without common characters in the different, existing groups. How the organisms would look under such circumstances is really not easy to imagine.

For Genetics which works with varieties, races, lines within any species or with cultivated plants and animals, the systematical units have only secondary importance. Several hypotheses on which DARWIN based his doctrine of natural selection Genetics has rejected. As the genetical opponents of DARWIN, especially in popularized works and lectures, have not always distinguished clearly between the doctrine of selection and those of descent and of evolution, all these doctrines have suffered. To illustrate this by an example I can refer to the words translated which appeared in one of our largest newspapers the day after the award of the Nobel prize to MORGAN in 1933, viz. "In comparison with the scientific work of MORGAN DARWIN's doctrines of descent and natural selection are mere bosh". As the geneticists have in silence allowed genetical spokesmen to discredit DARWIN's work to such an extent that all biological science suffers, it is I think high time that something be done, especially by men interested in Genetics to prevent further injury. Genetics itself is not invulnerable either.

The basis for the systematics' "genealogical" systems is, as mentioned, the species, i.e. that group of indivi

duals which is not to be divided into independent parts with respect to the individual characters and in which, then, all individuals are in the main alike, except of different sex, age, customs (morphae), monstrosities etc. In my paper (1935) quoted above I maintained that such a group is fully or completely limited and I added that the aim of a perfect systematical working is to prove the discontinuity between the different species and also between the other units as well. These units do not claim *a priori* to be anything absolute. As the units of the elder systematists have frequently been proved to be mixed and by that have been divided into two or more units, a systematist of the present time must resign, if his units meet the same fate due to more recent finds, more suitable characters, regroupings etc. The systematical catalogues, at least those which concern animals, are abundant in species, especially of more ancient date, which do not satisfy my definition above of a fully limited species. They are mostly based upon unimportant divergences in size, colours and other individually shifting qualities in an animal. They are reminiscences of a period of systematization whose days are past. No modern author accepts them. They distort a rational System, confuse the non-initiate, give trouble, and make difficulties for the expert. Nothing in the animal systematization is more urgent than a revision based upon uniform and rational principles.

Even if a species is correctly detailed and distinctly limited from other species, there are usually slight differences in one or the other quality of the population of the species in question. This fact has ever been confusing. Only by experience does one learn to discriminate characters suitable for systematical purpose from all the other details of the animal body. Such characters are often not of the same kind even within so closely related groups as the different families of beetles for instance. On account of the mentioned different details a species seems, sometimes at least, to consist of lines or races which, however, are not fully limited from each other, but show clear connecting links or intermediates. The same is true of most of the varieties and subspecies included in the systematical catalogues. But

some members of these two categories last mentioned have proved to be true species, especially among the geographical varieties and subspecies.

It is on the species that the whole Systematology of plants and animals has been built up. It is likewise on the species, i.e. on its races, that the physiological science which we call Genetics has raised its already imposing doctrinal system. It was, as known, by the discovery of the regularity in number and appearance of the different offsprings after crossing of two cultivated races of peas, that Genetics came into being. Still the rule prevails that only by crossing of lines, races, varieties etc. of a species which differ from each other in one, two or more respects Genetics is able to give us information, but only concerning the divergent qualities. As to the similar ones Genetics gives us no information at all, as little as to the characters of the species and to those of the other higher units. The information given by Genetics as regards the differences, is mostly written in symbolic idioms and upon a woven ground of hypotheses. Thus they are quite different from the results of systematical work with its ground free of every hypothesis. But in spite of that the information of Genetics is no less valid as evidence. There was a time when the atom was a mere hypothesis. Now the vestiges of the constituents of an atom can be photographed and, thus, objectively proved.

Some of the most important consequences of genetical explorations are the penetrating cell-studies and the dynamic of heredity. As just mentioned, this is restricted only to the differences. For that reason some genetic authors, for instance PLATE (1933) maintain that the similar qualities of crossed individuals have their gene-substance placed in a fixed combination, "Erbstock", outside the chromosomes where the genes for the differences are proved really to lie. By this a sharpened limit would be drawn between these two categories of qualities without correspondence in the reality. In Genetics there is already too much of hypotheses, and I think it better if we could be satisfied by one gene-hypothesis. In the chromosomes of *Drosophila* for instance there is plenty of space for genes corresponding to all similar characters of every systematical degree, this so much the

more as the number of the characters decreases considerably for every step upwards in the systematical series. We see in the bastards except at a certain dominance the differences between the parental gametes, i.e. between their genes, but we cannot expect, of course, to see anything where the genes are congruent in the copulating gametes.

Though it has been possible to cross even different species the results have not given any more definite rules, neither in the F_1 - nor in the F_2 -generation. It is known that some crossed species give mendeling F_2 , but for the most part it is not so. The F_1 -generation is mostly said to be intermediate, i.e. something between the two parent species. Such intermediate forms should henceforth breed constantly. From my own experience I can report on a crossing between two species, before not known to interbreed as far as I know, which already in F_1 gave a spalting offspring. Unfortunately the season was inconvenient for the pursuit of the breeding to the F_2 -generation. The crossing was fully spontaneous. In the summer of 1935 I saw on the margin of a glass-jar in my open kitchen window a copula of two small flies which seemed to be very odd. I caught the copula and put it into a jar with crushed banana pulp. After about a fortnight I killed and examined the two flies. The male was *Drosophila fasciata* MEIG. (= *ampelophila* LOEW or *melanogaster* SCHIRN.), the female was *Dros. funebris* F., both named according to ERVIN LINDNER: Die Fliegen der palaearktischen Region (in printing 1935). I had an expert on flies scrutinize my examination. Inspecting the jar, I saw some 70 eggshells on the dried crust of the pulp and transferred a little more than 50 larvae of different sizes to another jar with fresh banana pulp. A dozen larvae went soon afterwards into the pupal stage, and the rest perished, probably because of mould and fermentation in the pulp during the autumn. All the pupae hatched and gave offspring of three kinds to about the same number each, viz. one small of *fasciata*-type, one large of *funebris*-type and one intermediate in size, but more like a *funebris* in colour. Although this is only a simple preliminary experiment of small extent, it is evident that a spalting took place already in the F_1 -generation, giving the parental forms together with an intermediate one.

The difficulties with respect to the study of the relations of the

species-characters by crossing of species are considered to depend upon the intolerance of the gametic chromosomes with each other or with the egg-cytoplasm by the zygotal genesis. It is a fact, long ago known through the works of KÖHLREUTER and GÄRTNER, that the more distant in systematical respect two individuals are, the more difficult is to cross them. There was a time when for a group of individuals the right to bear a specific name was decided by the wanting capacity of its representatives to give offspring by crossing with representatives of another species group. One thought one had found by that a decisive difference between species and variety and undertook, because of that, alterations in the systematical arrangements which, however, led to obvious errors. Due to his extensive explorations DARWIN (Origin of sp.) settled in the first place that individuals of different species either were not to bring to copulation, or, when it succeeded, did not produce offspring or produced sterile ones, but that exceptions to this rule were to be found; in the second place that individuals of varieties of the same species crossed without difficulty and gave fertile offspring, but that even in this case exceptions occurred. Because of these results DARWIN emphasized that the above mentioned criterion for an authorisation of a species could not be accepted. After the appearance of Genetics the situation remains unaltered in the main, the exceptions, however, have increased in number within both categories, especially concerning plants.

Here I may accentuate that the genetical texts and handbooks do not discriminate distinctly enough between some matters appertaining hereto, probably because of their wrong general conception of the species as a jumble of environmental modifications and gene-governed constituents. In the first place I think it necessary to discriminate between plants and animals in treating of such intricate questions as crossing of species. Metazoic animals in their sexual stage are perfect individuals with finished growth, very little dependent on environmental factors on account of the power of voluntary locomotion. The species of most animals include, thus, individuals of a more concentrated bodily structure with internal organs for vital functions and with an organisation of their whole body which we must admit to be higher than in plants. As little as the relations among *Protophyta* and *Protozoa* decide our conception of reproduction

among higher plants and animals, we can presume congruence between plants and animals with respect to crossing-results. Moreover it must be clearly stated in the textbooks that, by crossing of varieties, it is understood *varieties of the same species*. This is by no means self-evident for a novice.

A comparison of species-crossing with common race-crossing looks to a systematist like an attempt to compare two incommensurables. It must, I think, be admitted that two lines, races, varieties etc. of a species are nearly related to each other and can, because of this fact, *a priori* be considered rather accordant with respect to their whole constitution. They bear every sign of being parts of one and the same quantity. Two different species, inclusively their lines, races, varieties etc. must, on account of their self-dependent systematical position respectively, be considered as different quantities. This is strengthened by the simple fact that it has never succeeded in altering a recognized species of plants or animals, mono- or pluricellular, into another recognized one. In such circumstances it is, of course, vanity to expect accordant results of race- and species-crossing.

Through his own and others' experiences in regard to cultivated organisms DARWIN found that the individuals of a race showed different qualities, great or small, which reappeared in the offspring (were hereditary), sometimes in increased degree. Although he admitted, at least as to animals, that this individual variation was not so striking in living nature, its existence could not be denied. It led to the origin of different varieties which, if more pronounced and fixed, he called "incipient species". To him the disintegration of the wild species into a mass of varieties was, as he said himself, a question of greatest importance for the establishment of his doctrine of natural selection. The more variation, the more points for the attack of the selection. This may be quite logical. But how does it agree with the facts? At least concerning animals a comparison of the number of species with that of the recognized varieties within the same group proves that the latter one amounts to a trifling minority. For instance within the family of *Dytiscidae* with about 2100 species the number of such varieties amounts to 4

per cent. Within other groups of beetles which I have studied the minority is still smaller. About the same conception of the trifling number of varieties in comparison with the corresponding species I obtained from my previous studies of Reptiles and Echinoderms from different parts of the world. I am convinced that the relations will be the same, in the main at least, with respect to all animals, possibly Mollusca and some others excepted in which the conditions are not to be compared by reason of the fact that the specific characters lie in the shells or in other dead products of secretion in the genesis of which the environment of the individual plays a greater rôle than elsewhere.

The varieties included in the systematical works are often put to species with a wide-extended geographical distribution. Such varieties have in some cases by nearer inspection of new material proved to be independent species. The greater number of the elder varieties are, indeed, merely reminiscences from a time when small aberrations in colour and the like played a rôle in Systematics. As nowadays a single difference in colour not in combination with any other difference of the specific characters do not legitimate a new variety, the disappearance of the old colour-varieties is only a question of time. But all remaining varieties cannot be certain of retaining their separate place within the respective species. According to the advance of Systematics, to better methods of investigation and to more material, some of these varieties, mostly known in a single representative from a distant part of the earth, have even proved to be independent species. Thus, at the same time as the number of species increases in this way, the multitude of varieties supposed by DARWIN changes into an unimportant minimum. By estimation according to the same standard, the ratio between species and varieties of animals just given has, of course, been similar almost in all times, even in that of DARWIN. I should think that the same is the case also in plants. If not, this circumstance can have no influence upon animal Systematics or upon the judgment of these questions within Zoology. Moreover, the number of known animal species from the whole earth is so enormously greater than that of plants, on account of which it ought to be self-evident, I think, that the prevailing state among animals may not be set aside by arrangement of laws within Biology and Genetics as has been done in many cases.

In my account, just given, one might perhaps object that DARWIN may have made a mistake in his calculation of the number of the real varieties, but the species, irrespective of the varieties, are not uniform but complexes of biotypes, "pure lines", or whatever they may be called. True, but as little as this circumstance finds expression in a more visible variation, so little it gives the rich multitude of points presumed by DARWIN for an attack by the natural selection. Plenty of instances for this fact within animals and plants are given by those species whose characters do not vary in a noteworthy degree on account of their presumable homozygoty in both animal sexes, except the sex-linked ones. Species of this kind we have, as known, even from early palaeozoic time in the *Cephalopod* and *Brachiopod* groups which have existed, without alteration, during enormous spaces of time in comparison with the lifetime of their representatives. Such species which do not vary, although they may have an extended geographical distribution, DARWIN seems to have been inclined to put aside as being useless for the doctrine of natural selection. They are, however, too numerous to be neglected and their existence must have an explanation by a doctrine with that extensive aiming which the doctrine of selection had at the time of its emergence.

The other great group of species which shows a distinct power of variation, DARWIN took as basis for his doctrine in question. A rough estimation, I admit, of the number of such species within the adepagous and some of the polyphagous families of beetles according to present Systematics shows that this number makes a minority of all species of the same families. It is not difficult to see that the same ratio prevails within the whole Order of beetles. Since the number of species of beetles far exceeds that of all other known species of animals in the world together, it is evident that DARWIN's doctrine of natural selection rests on a minority of animal species whose representatives show sufficient power of variation as basis for the working out of new species according to DARWIN's doctrine. One will perhaps object to that as follows: what now prevails is changeable; species, now constant, may have been variable in an earlier period. Possibly, but hence it follows that the now varying species can be constant. There is no rest for the thought that

the ratio between the constant and varying species changes by that.

From what DARWIN says, it appears that an abundant occurrence of small individual variations within the species was a fundamental supposition for his doctrine of selection. He thought that these variations could be increased as in cultivated forms and by means of selection, enforced by the struggle for life, also could be directed and give rise to varieties from which true species should arise at last. Varieties and species would in this way be confluent, and the restriction of a species would be arbitrary, as his words read. It seems to me evident that sufficient experience in hard systematical work would have been very useful for DARWIN. Now he seems to consider the variety as the primary and the species as the secondary stage. If so, the varieties must nevertheless have a starting-point, a form from which they take their origin and which is subjected to a considerable variation. It would then be another species. But, according to DARWIN's assumption mentioned above, all species should be of the same variable quality. The living world would in this way really flow together into a few very little differentiated bulks, separable from each other only by the great lines which distinguish the higher groups in the systematical series, an opinion which ERNST HAECKEL probably shared, since he would ascribe realness only to the *phyla* of animals and plants. Such conceptions are, however, inconsistent with a scientific system for which the living world is an ordered totality¹ of real constituents from the base to the top which are clearly distinguishable from each others, at the base (*viz.* the species) sometimes not without considerable difficulty.

If we overlook the statements made, we shall find that DARWIN has made exceptions to the rule. The variations and the varieties do not play the dominant rôle in nature which he has awarded to them as a basis for his doctrine of natural selection. By that it is even shown that DARWIN made a mistake when he contended that a species should be variable in its essence. Only

by heterogeneity or as reaction against altered environmental conditions does the variability arise.

The restriction with regard to the very material for its working to which natural selection has submitted according to the above stated, needs not, I think, to cause a total rejection of the doctrine of natural selection. It is valid to a certain degree. As an eliminating factor by inferiority its supposed effect remains undisturbed. In the main, i.e. as a factor in the origin of new species this doctrine on the other hand has only historical interest. As a confusion of the doctrine of selection and the doctrine of descent occurs in public opinion, I think it is of importance for biological science, non the less for real tactical reasons, that the biological texts and handbooks may clearly and distinctly establish that the doctrine of descent does not stand or fall with DARWIN's doctrine of natural selection. The veneration which we owe the great thinker and pioneer remains undisturbed. By his contribution to the definite breaking through of the doctrine of descent and by that to the deliverance of biological thinking from misconceptions of an unconnected, religious content, DARWIN has sealed the emancipation of the human spirit. The value of this is not lessened by an open admission of the insufficiency of the doctrine of selection in solving the problem of the origin of species.

This problem remains as unsolved as before. Rather it seems that the raising of those elementary groups of individuals called species, cannot be referred to a single principle, but may be considered to have different causes. Likewise the ways seem to be divergent which the individuals of a group have to take in order to become sufficiently different from individuals of other groups. This differentiation seems rather to be a resultant of different forces on account of which different results are to be expected. It is especially the conserving force of the heredity, i.e. of the totality of the gene-substance and the forces of every kind influencing this substance which stand in the foreground. One must distinguish between the milieu of the individual and that of the gene-substance, i.e. the contents of the developing zygotic cell. Only the latter has importance. The process of differentiating lasts as long as the milieu of the genes presses.

The variability of a species cannot for that reason be a constant phenomenon as DARWIN thought. On the other hand, the power of heredity is constant within a species under unaltered conditions to the genes. This is, of course, self-evident, since the genes are transported over to the filial generation by the gametes. As soon as equilibrium between the forces has been reached, the new form remains constant until some perturbation arises again. These are caused in a natural way by irregularities in the chromosomal dynamic, by amphimixis (for instance: species-crossing) by strong fluctuations in climate, moisture, aridity, chemical composition of the alimentation and so on.

The power of constant heredity, except by perturbations, is an attribute of the individuals of a species which corresponds in the most intimate manner with their power of evolution from egg to full-grown organism, i.e. ontogenetic evolution. In my often quoted paper (FALKENSTRÖM 1935) I have also emphasized the constancy of the species under unaltered conditions as necessary for phylogenetic evolution as well as for the doctrine of descent which we have learned to know by Palaeontology and by the Systematology of plants and animals. I said there also that constancy of heredity with consequence of course, of constancy of species is "*conditio sine qua non*" for phylogenetic evolution, having especially in mind the higher organized forms of plants and animals. Life on our earth can be thought of even under perpetual alterations of the living things, but in no wise such a highly differentiated life as we have and, according to the palaeontological documents, have had from their beginning without the fixed points of support which constant species can give to the process of differentiating.

B. SPECIES-CROSSING IN *Deronectes* (BEETLES)

I. Sweden and neighbouring countries

I mentioned just above intercrosses as causes for perturbations and failure of constancy within the species. Besides the established results of crossing of our cultivated plants and animals and the trials of crossing wild organism in the laboratories, there have lately been known intercrossings of plants in nature, sometimes causing the primary species to be displaced, fully or partly. In animals nothing like that was known until in 1932 I reported (FALKENSTRÖM 1932) on certain explorations which I had begun in 1930. With reference in other respects to this report and to two later papers (FALKENSTRÖM 1933 and 1936) I shall now report more in detail on the results of crossing between representatives of some species of beetles, and point out intercrossing in masses of similar beetles in nature with obvious influence on the fauna.

By the discovery of a loose wing-case among plant-remains from the shore of Mälär Lake in 1926, my attention was called to the fact that we must have a species of *Deronectes* in this region hitherto unknown in our country. I supposed that it was *D. elegans* PANZ. known in Central and West-Europe. In spite of repeated searchings I was not able to find a living specimen, but later on in 1930 I succeeded in finding one of the wanted representatives in another part of Mälär Lake in the neighbourhood of historical "Stäket". It was a female, at once distinguished from *elegans* by its dark ventral side. The following day it began to set eggs from which I bred a great series of larvae. At the same time I had at home two pairs, ♂ and ♀, of *Deronectes depressus* F. which I obtained from West-Sweden a year ago. It happened that the one female of these pairs also set plenty of eggs in her jar. By that I got two parallel series of larvae, being thus easily able to perceive the striking differences between the two kinds of larvae. After the passage of the pupal stages I got from the one series lots of uniform *depressus* and from the other likewise uniform beetles, not of *elegans*, but of a new species which I called *latescens*. The complete constancy of these two species in all their ontogenetical stages I have proved during 5-6 years by yearly interbreedings of brothers and sisters, alternating by back-crossing with the parent-

generation, always, of course, under strongest individual control. It is evident that I have worked with biotypes of both species. By examination of FABRICIUS' type-material of *depressus* (only two specimens, ♂ and ♀ remain) I have seen that my specimens of this species are in every respect fully identical with FABRICIUS' specimens and that his specimens are likewise uniform. According to his words he established the species on Swedish material.

Besides on the shores of Lake Mälär, i.e. in fresh water, I have found *latescens* in a distant bay of the Baltic Sea, "Säbyviken", where the water is slightly brackish (*Fucus vesiculosus* and other fucoid plants are sometimes to be found as wracks). The species seems, to be a form of larger open waters where the specimens dwell among green-algae on loam-bottom adjacent to a coarse stony shore. *D. depressus*, on the contrary, is said by GYLLENHAAL, *Insecta suecica* 1808, to occur "in aquis passim, praesertim in fluviis, inter Charas" and by C. G. THOMSON, *Skandinaviens Coleoptera* 1859, translated, "between Confervae in running water". My material of this species was taken in 1929 from a small lake at Skövde (Vestergötland) according to information received. I believed for a long time that I myself had found this species at some distance from here in a bay of the Baltic Sea, "Vadviken", at the baths of Dalarö where the water is slightly brackish, which, however, proved to be erroneous. That place had a sandy shore without any vestige of plants in the water. As the animal occurred in numbers, there were probably plants in the deep.

By breedings of the larvae having fully recognized the never-ceasing uniform appearance of the hatched beetles of *depressus* and *latescens*, respectively, I found that my material of the supposed *depressus* from Dalarö was distinctly different, since about half of the number of specimens was in the main like *depressus*, the rest on the contrary like *latescens*. Had I not seen such clear and constant differences in the whole ontogeny (*vide* FALKENSTRÖM 1933) of these two species, I should not have devoted more time to further investigations of the matter. Now I resorted to crossing experiments. I brought together ♂ of *depressus* and ♀ of *latescens*, ♂ of *latescens* and ♀ of *depressus*, two pairs of each. The used specimens were bred in the previous year under strong individual control and each kept isolated until the experiments were to be made. These succeeded beyond expectation and the result was in its principal traits as follows: a

successful crossing took place in both directions without an increased mortality during the development, the larvae and the hatched beetles showed a shifting composition of the specific characters, and finally, the beetles hatched were exactly of the same appearance as my supposed *depressus* from Dalarö. Because of this result I considered as proved that a population of bastards between *depressus* and *latescens* was living in Vadviken at Dalarö. For the same reason there was, I thought, no cause for delaying further the publishing of the new species *latescens* which could claim to be as well-established as was possible. I maintain this view now just as decidedly, having during the past years seen new generations of the same constant species-type arising from my, especially with regard to *depressus*, strongly interbred specimens.

I have seen a similar population in a collection from the neighbourhood of Västerås taken by Cand. J. SELLMAN probably in Mälars Lake. As to the rest, it is very seldom that one finds, in collections from Sweden, Finland and Denmark, the mentioned species pure. There are usually mere bastards, recognizable by certain details in their colour-pattern which resemble closely those of *latescens*. It is such bastards which have hitherto usually passed for *depressus* in the northern countries. To get an adequate idea of these intricate matters, it is necessary to enter more fully into particulars than is usual in systematic works.

In my paper (FALKENSTRÖM 1932) I gave a comparative table of the main morphological differences — about 20 — in imagines of *depressus* and *latescens* together with drawings of some important characters and photos of the beetles and larvae. In a later paper (FALKENSTRÖM 1933) I showed by means of descriptions, numerous drawings and photos proving that all stages of the two species during the whole entogenetical development exhibit in comparable parts numerous distinct differences, partially very striking ones. Referring for the rest to these two papers, I will confine myself now to some differences of the fullgrown beetle and the third larval stage which are easily accessible for comparison and, by their proved constancy, suitable for the estimation of the crossings performed.

a. Imaginal stage

I shall begin with the imaginal forms, i.e. the beetles of the pure species and only treat of the general colour and some colour patterns on the wing-cases. In *depressus* the yellowish-brown ground of the wings is barely conspicuous on account of the confluent black longitudinal lines. The black sutural line begins with a triangular dilatation at the base and continues to the very apex of the wing. Close to the black suture and usually confluent with it, the 1st line (l_1), lies on each wing and ends with a small tooth-shaped dilatation at a short distance before the apex. The 5th line (l_5) is coalescent with the 4th line and others and extends as a black agglomeration to the wing-base. On account of a partial reduction of the 2d and 3d lines the ground becomes visible as two very small, longitudinal, yellowish-brown spots, the one (ss_1 = sutural spot) at the base, the other (ss_2) just behind the middle of the wing. At the apex there is a small, irregular, yellowish spot (as = apical spot). At the margin of the wing there are three yellowish spots (ma = marginal spots), one at the shoulder (ms_1), one just before the middle (ms_2) and the third just behind the middle (ms_3). In *latescens* the ground is straw-coloured and much more visible since the black lines are narrow, not confluent and partially more reduced. The suture is likewise black, of the same shape and reaching the apex. Line l_1 begins at a distance behind the basal sutural triangle, ending, not dilated, on the level of the other black lines, thus, far from the apex. Line l_5 extends alone to the wing-base. Ss_1 and ss_2 are much enlarged and behind the latter there is often another yellow spot (ss_3) confluent with the large triangular apical spot (as). The three marginal spots (ms_1 , ms_2 and ms_3) are also much enlarged. The correctness of my description, just given, will I think be duly verified by a glance at the photos of the animals (FALKENSTRÖM 1932) and at those of their wing-cases on Plate I figg. 1-4 of this report. On living specimens of *latescens* in water, these 6-7 yellow spots appear as a highly marked difference from the barely visible spots of *depressus*.

The 8 specimens, used for the crossing-experiments in 1931, were with regard to colour and colour pattern typical according to my description above, except that ss_3 was to be found only on two of the specimens of *latescens*, viz. in series A and C. I have called the four crossing pairs and their larvae series A, B, C and D.

Ser. A = *depressus* ♂ (*depr.* B₁₀ 1930) × *latescens* ♀ (*Der.* X₄ 1930), hatched of larva from "Säbyviken".

Ser. B = *latescens* ♂ (*Der.* X₉ 1930, hatched of larva from "Säbyviken") × *depressus* ♀ (*depr.* B₂₆ 1930).

Ser. C = *depressus* ♂ (*depr.* B₁₁ 1930) × *latescens* ♀ (*lat.* 5 1930, belonging to the second generation of bred specimens from "Stäket" in that year).

Ser. D = *latescens* ♂ (*lat.* 8 1930, belonging to the same hatch as the ♀ in Ser. C) × *depressus* ♀ (*depr.* B₄₅ 1930).

Series A and C are thus *depr.* ♂ × *lat.* ♀; series B and D on the other hand *lat.* ♂ × *depr.* ♀. In the beginning the series of larvae were large, but they were soon restricted to 20–30 specimens of each, since the time did not allow more for the control's sake. In every series I let the first laid eggs deliver the beetles. Below detailed descriptions of the hatched beetles follow.

Series A (*depr.* ♂ × *lat.* ♀). F₁: A₁ ♀, A₂ ♀, A₆ ♀, A₇ ♀, A₉ ♀ and A₁₀ ♀

A₁: ground yellow, *l*₁ begins, narrowed, at the sutural triangle, ends without dilatation near to apex, *l*₅ and *l*₄ coalescent at the base, *ss*₁ enlarged as in *lat.* *ss*₂ as in *depr.* *ss*₃ wanting, *as* a little enlarged, *ms*₁ and *ms*₂ ditto, *ms*₃ as in *depr.*

A₂: ground yellow, *l*₁ in front and behind as in *lat.* *l*₅ in front swelled, but extends alone to the base, *ss*₁ as in *lat.* *ss*₂ a little larger than in *depr.* *ss*₃ wanting, *as* as in *lat.* *ms*₁ and *ms*₂ next to *lat.* *ms*₃ next to *depr.* but a little enlarged.

A₆: ground slightly brownish-yellow, *l*₁ in front square, beginning at a very short distance from the sutural triangle, ending on the one wing as in *lat.*, on the other reaching near to apex, without dilatation, *l*₅ in front swelled, reaching alone to the base, *ss*₁ a little enlarged, next to *depr.*, *ss*₂ as in *depr.*, *ss*₃ wanting, *as* next to *lat.* *ms*₁, *ms*₂ and *ms*₃ a little enlarged but next to *depr.*

A₇: ground slightly brownish-yellow, *l*₁ in front slightly narrowed, next to *depr.*, ends behind as a line like *lat.*, but continues as a row of small spots near to apex, *l*₅ is in front on the one wing nearly, and on the other quite coalescent with *l*₄, reaching swelled to the base, *ss*₁ next to *lat.*, but is bisected by a black spot, *ss*₂ as in *depr.*, *ss*₃ is indicated by an interruption of the 2nd line, *as* next to *lat.*, *ms*₁ and *ms*₂ enlarged, but next to *depr.*, *ms*₃ next to *lat.*

A₉: ground slightly brownish-yellow, *l*₁ in front narrowed as in

depr., ending behind as a distinct line in the same way as in *lat.* but continuing on the one wing as a shadowed line, on the other as a row of vague spots near the apex, l_5 in front coalescent with l_4 reaching, swelled, to the base, ss_1 next to *depr.* but a little enlarged, ss_2 as in *depr.*, ss_3 indicated by an interruption of the 2nd line, *as* next to *lat.* but a little smaller, ms_1 and ms_2 next to *depr.* but a little enlarged, ms_3 , wanting.

A₁₀: ground slightly brownish-yellow, l_1 in front like *depr.*, behind even like *depr.* but the dilatation at the end is small and pale, l_5 in front coalescent with l_4 at the base, ss_1 next to *depr.* but a little enlarged, ss_2 as in *depr.*, ss_3 wanting, *as* as in *depr.*, ms_1 and ms_2 a little enlarged but next to *depr.*, ms_3 as in *lat.*

Series C (*depr.* ♂ × *lat.* ♀). F₁: C₁ ♀, C₂ ♂, C₄ ♀, C₅ ♀ and C₇ ♀.

C₁: ground slightly brownish-yellow, l_1 in front square, beginning near to the sutural triangle, behind most like *depr.* but not so distinct in the part behind the other black lines, l_5 coalescent with l_4 at the base, ss_1 and ss_2 like *depr.*, ss_3 wanting, *as* triangular but smaller than in *lat.*, ms_1 and ms_2 as in *depr.*, ms_3 next to *depr.*

C₂: ground brownish-yellow, l_1 in front as in *depr.*, behind next to *depr.* but the dilatation without a tooth, l_5 coalescent with l_3 and l_4 at the base, ss_1 and ss_2 as in *depr.*, ss_3 wanting, *as* nearly like *depr.*, ms_1 , ms_2 and ms_3 as in *depr.*

C₄: ground slightly brownish-yellow, l_1 in front as in *depr.*, behind reaching near to apex but in the hindmost part continued as dark points, l_5 coalescent with l_3 and l_4 at the base, ss_1 as in *depr.*, ss_2 next to *depr.* though a little enlarged, ss_3 wanting, *as* next to *depr.*, ms_1 , ms_2 and ms_3 enlarged, but next to *depr.*

C₅: ground yellow: l_1 in front next to *lat.* but the distance of the square end to the triangle is short, behind as in *lat.*, l_5 reaches, swelled, to the wing-base, ss_1 next to *lat.* though smaller, ss_2 as in *depr.*, ss_3 wanting, *as* like *lat.*, ms_1 next to *lat.*, ms_2 as in *depr.*, ms_3 near to *lat.*

C₇: ground slightly brownish-yellow, l_1 in front square, begins close to the triangle, ends as a line on the level of the other black lines, but continues as a row of black points near to apex, the points decreasing towards the end, the contrary thus to a dilatation, l_5 extends alone, but swelled, to the wing-base, ss_1 and ss_2 as in *depr.*, ss_3 wanting, *as* triangular, next to *lat.*, ms_1 next to *lat.* but smaller, ms_2 as in *depr.*, ms_3 enlarged, intermediate.

Series B (*lat.* ♂ × *depr.* ♀). F₁: B₁ ♂, B₂ ♀, B₃ ♀, B₄ ♀, B₅ ♂ and B₆ ♂.

B₁: ground nearly brownish-yellow, *l*₁ in front and behind as in *depr.*, *l*₅ coalescent with *l*₃ and *l*₄ at the wing-base, *ss*₁ and *ss*₂ as in *depr.*, *ss*₃ wanting, *as* triangular, but smaller than in *lat.*, *ms*₁, *ms*₂ and *ms*₃ enlarged, intermediate.

B₂: ground brownish-yellow, *l*₁ in front square, beginning close to the triangle, behind next to *depr.* but without a tooth at the end, *l*₅ coalescent with *l*₄ at the wing-base, *ss*₁ and *ss*₂ next to *depr.*, *ss*₃ wanting, *as* next to *depr.*, *ms*₁, *ms*₂ and *ms*₃ larger than in *depr.*, intermediate.

B₃: ground yellow, *l*₁ begins close to the triangle, but has the exterior edge pointed at the end (the contrary to *depr.*), ends on the level of the other black lines, but continues as a shading near to apex, *l*₅ is coalescent with *l*₄ and *l*₆ at the wing-base, *ss*₁ as in *depr.* though enlarged, *ss*₂ as in *depr.*, *ss*₃ wanting, *as* nearly as in *lat.*, though smaller, *ms*₁ and *ms*₂ larger than in *depr.*, intermediate, *ms*₃ nearly as in *lat.*

B₄: ground slightly yellow-brown, *l*₁ begins square at a short distance from the triangle, continues near to apex, but towards the ends is continued as points, the very end being a transversal, oval spot, imitating a hook or tooth, *l*₅ reaches on the one wing, swelled, alone, on the other confluent with *l*₄ to the wing-base, *ss*₁ and *ss*₂ as in *depr.*, *ss*₃ wanting, *as* nearly as in *lat.*, though a little reduced at the suture, *ms*₁ and *ms*₂ next to *depr.*, though enlarged, *ms*₃ nearly as in *lat.* though a little reduced.

B₅: ground slightly brownish-yellow, *l*₁ begins on the one wing narrowed, on the other squarely close to the triangle, ends without dilatation near the apex, *l*₅ reaches, dilated, alone to the wing-base, *l*₄ touches *l*₅ at a distance from the base on the one wing, *ss*₁ and *ss*₂ as in *depr.*, *ss*₃ wanting, *as* triangular, but is much smaller than in *lat.*, *ms*₁ and *ms*₂ are a little enlarged, most like those of *depr.*, *ms*₃ next to *lat.*

B₆: ground yellow brown, *l*₁ in front square, beginning close to the triangle and continues, after a short interruption on the level of the ends of the other black lines on the one wing, near to apex with a tooth-shaped dilatation at the end, *l*₅ reaches, dilated and touching the united *l*₃ and *l*₄, to the wing-base, *ss*₁ and *ss*₂ as in *depr.*, *ss*₃

wanting, *as* subtriangular and smaller than in *lat.*, *ms*₁, *ms*₂ and *ms*₃ as in *depr.*

Series D (*lat.* ♂ × *depr.* ♀). F₁: D₁ ♂, D₂ ♂, D₃ ♂, D₄ ♂, D₅ ♀, and D₆ ♀.

D₁: ground brownish-yellow, *l*₁ beginning at the triangle, square on the one wing, narrowed on the other, continues nearly to apex, towards the end partially interrupted, the very end forming a round point, *l*₅ reaches alone and dilated to the wing-base, but is on the one wing united with *l*₄, *ss*₁ and *ss*₂ as in *depr.*, *ss*₃ wanting, *as* subtriangular, next to *depr.*, *ms*₁ enlarged, intermediate, *ms*₂ and *ms*₃ next to *depr.*

D₂: ground slightly brownish-yellow, *l*₁ begins square close to the triangle and continues nearly to apex, ending, pointed, after a rounded dilatation, *l*₅ reaches, dilated, alone to the wing-base, *ss*₁ and *ss*₂ as in *depr.*, *ss*₃ wanting, *as* triangular, but smaller than in *lat.*, *ms*₁ enlarged but next to *depr.*, *ms*₂ as in *depr.*, *ms*₃ ditto, but a little enlarged.

D₃: ground brownish-yellow, *l*₁ in front and behind as in *depr.*, *l*₅ coalescent on the one wing with *l*₄, on the other with the rest of *l*₆. reaches, dilated, to the wing-base *ss*₁ and *ss*₂ as in *depr.*, *ss*₃ wanting, *as* as in *depr.*, *ms*₁ and *ms*₂ intermediate, *ms*₃ irregular, on the one wing separated from the yellow margin by a black line, on the other enclosing a black spot.

D₄: ground slightly brownish-yellow, *l*₁ begins, nearly square, close to the triangle and continues near to apex, ending slightly dilated, *l*₅ coalescent with *l*₄, reaches, dilated, to the wing-base, *ss*₁ and *ss*₂ as in *depr.*, *ss*₃ wanting, *as* triangular, intermediate, *ms*₁ enlarged but next to *depr.*, *ms*₂ and *ms*₃ next to *depr.*

D₅: ground brownish-yellow, *l*₁ in front as in *depr.*, continues, exceptionally thick, nearly to the very apex, ending with a slender dilatation, forming, on the one wing, a tooth, *l*₅ and *l*₄ coalescent at the wing-base, *ss*₁ and *ss*₂ as in *depr.*, *ss*₃ wanting, *as* as in *depr.*, *ms*₁ and *ms*₂ a little enlarged but next to *depr.*, *ms*₃ as in *depr.*

D₆: ground yellow, *l*₁ in front square and beginning at a short distance from the triangle, behind nearly like that of *lat.*, but with a black spot on the one wing on the place, where the line ends with a dilatation in *depr.*, *l*₅ reaches, dilated, alone to the wing-base, *ss*₁ next to *lat.*, *ss*₂ as in *depr.*, *ss*₃ wanting, *as* as in *lat.*, *ms*₁, *ms*₂ and *ms*₃ next to *lat.*, though a little smaller.

For the sake of surveyability I have put together these detailed

descriptions to Table 1. Concerning the abridgements used, they will, I hope, be fully explained by the preceding text, especially that under the descriptions of the true species (See p. 234). With "new" I mean a comparatively great divergence from one or both species, which could sometimes be called intermediate. I have, however, not distinguished between such forms and really new ones, as the former appear just as remarkable. In the literature there are doubtless many misuses of the indication "intermediate", since every degree between the two poles is often understood, probably by mere

TABLE 1. PARENTS IN SER. A AND C: *depr.* ♂ × *lat.* ♀, IN SER. B AND D: *lat.* ♂ × *depr.* ♀.

(Abbreviations: = *de*, = *la* — as in *depr.*, as in *lat.* resp. *in.de* — near to *depr.*, *n.la* — near to *lat.*)

Number F ₁	Ground colour	I ₁ in front	I ₁ behind	I ₆	ss ₁	ss ₂	ss ₃	as	ms ₁	ms ₂	ms ₃						
A1	= la	= de	new	= de	= la	= de	= de	new	n	la	n	la	= de				
A2	= la	= la	= la	new	= la	n	la	= de	= la	n	la	n	la	n	de		
A6	n	de	new	new	new	n	de	= de	= de	n	la	n	de	n	de		
A7	n	de	n.de	new	new	new	= de	new	n	la	n	de	n	de	n	la	
A9	n	de	= de	new	= de	n	de	= de	new	n	la	n	de	n	de	new	
A10	n	de	= de	n	de	= de	n	de	= de	= de	n	de	n	de	= la		
C1	n	de	new	n	de	= de	= de	= de	n	new	= de	= de	n	de	n	de	
C2	= de	= de	n.	de	= de	= de	= de	de	n	de	= de	= de	= de	= de	= de		
C4	n	de	= de	new	= de	= de	n	de	= de	n	de	n	de	n	de	de	
C5	= la	n	la	= la	new	n	la	= de	= de	= de	n	la	la	= de	n	la	
C7	n	de	new	new	new	= de	= de	= de	n	la	n	la	= de	new			
B1	n.	de	= de	= de	= de	= de	= de	= de	new	new	new	new	new	new	new		
B2	= de	new	n	de	= de	n	de	= de	n	de	new	new	new	new	new		
B3	= la	new	new	= de	n.	de	= de	= de	n.	la	new	new	n.	la			
B4	n	de	n.	la	new	new	= de	= de	= de	n.	la	n	de	n.	de	n	la
B5	n.	de	n	de	n	de	new	= de	= de	= de	new	n	de	n	de	n	la
B9	= de	new	n	de	n.	de	= de	= de	= de	new	= de	= de	= de	= de	= de	= de	
D1	= de	new	new	new	= de	= de	= de	n.	de	new	n	de	n	de	n	de	
D2	n.	de	new	new	new	= de	= de	= de	new	n.	de	= de	n.	de	n	de	
D3	= de	= de	= de	= de	= de	= de	= de	= de	= de	new	new	new	new	new	new	new	
D4	n.	de	n.	de	n	de	= de	= de	= de	new	n	de	n	de	n	de	de
D5	= de	= de	n.	de	= de	= de	= de	= de	= de	= de	n.	de	n	de	n	de	= de
D6	= la	= la	n.	la	new	n.	la	= de	= de	= la	n.	la	n	la	n	la	la

convenience. I have endeavoured to estimate the different characteristics as objectively as possible, which is, indeed, facilitated the more one enters into the particulars. Then, the characteristics used for comparison of the true species appear as very large divergences, allowing a sufficient marginal to the usual variation to be noted in every respect, provided that one is well acquainted with the matter.

As C_2 seems very near to being a pure *depr.* it may be noted that its posterior fore-claw on the left leg is like that of *lat.* but on the right leg that of *depr.*

From this table and perhaps even more from the descriptions of the specimens, it may be gathered that the bastards, as I have held forth before, show mostly a composition of the characters in question quite contrary to an intermediate heredity, equivalent with a state of the bastards in the middle between the two parental species. Nor is a dominance of any of these characters suggested. Very little of mosaic heredity is indicated, since the characters are, in the main, symmetrical on both wings. On the other hand it seems as if the individuals used would have had different power to let the specific characters of their species reappear in the offspring. Finally and with regard to the fact that the crossed animals in question belong to stocks which have during several generations after the crossing-experiments, under strong interbreeding within in the pure species given a progeny without any alteration whatever of the specific characters used, I cannot find that heterozygoty within one or both parents is relevant to explain the shifting appearance of the bastards, a statement often attempted in irregular crossing-trials.

In the following I shall give descriptions of the corresponding characters in the collection of *Deronectes*, taken in 1922 at Dalarö (Vadviken) which I supposed, at first, to be *depressus* but which are, In am sure, a lot of bastards between *lutescens* and *depressus*. The collection has been reduced a little in number by gifts and preparations. The remainder are numbered and marked as follows:

1a ♂: Ground slightly brownish-yellow, l_1 , beginning at the triangle squarely on the one wing, narrowed on the other, continues as a line to the level of the other black lines and after that shortly interrupted on the one wing, partly broken up to points on the other, continuing near to apex without dilatation, l_5 reaches, enlarged and on the one wing connected with l_4 , to the wing-base, ss_1 as in *depr.* but enlarged,

ss_2 next to *lat.*, ss_3 wanting, *as* triangular but smaller than in *lat.*, ms_1 and ms_2 next to *lat.*, ms_3 next to *depr.*

2b ♂: Ground slightly brownish-yellow, l_1 in front pointed in the exterior edge and beginning at a short distance from the triangle, continuing behind only to the level of the other black lines, l_5 as in *lat.*, ss_1 as in *depr.*, ss_2 intermediate, ss_3 only on the one wing indicated by an interruption of the 2d line, *as* in *lat.*, ms_1 as in *lat.* but minor, ms_2 nearly to *depr.*, ms_3 near to *lat.*

3c ♂: Ground slightly brownish-yellow, l_1 begins square at the triangle, behind as in *depr.*, l_5 reaches alone to the wing-base, enlarged on the one wing, ss_1 a little larger than in *depr.*, ss_2 about as in *depr.*, ss_3 wanting. *as* as in *depr.*, ms_1 next to *lat.*, ms_2 enlarged but next to *depr.*, ms_3 on the one wing as in *depr.*, on the other nearly so.

4d ♂: Ground yellow, l_1 beginning pointed on the outer edge at a distance from the triangle, continues near to apex with a slight dilatation but without a tooth, l_5 reaches swelled alone to the wing base, but on the one wing l_4 shows the same form, ss_1 as in *depr.*, ss_2 next to *depr.*, ss_3 wanting, *as* nearly as in *depr.*, ms_1 and ms_2 next to *depr.* but larger, ms_3 next to *lat.*

5e ♂: Ground slightly brownish-yellow, l_1 in front square, beginning at a different distance from the triangle on the two wings, behind as in *lat.*, l_5 as in *lat.*, ss_1 enlarged but next to *depr.*, ss_2 next to *lat.* but smaller, ss_3 wanting, *us* as in *lat.* but a little smaller, ms_1 and ms_2 next to *lat.* but smaller, ms_3 next to *depr.*

6f ♀: Ground yellow-brown, l_1 begins like *lat.* at a distance from the triangle but rounded off at the end on the one wing and pointed on the other, continues as a line to a little before the ends of the other black lines, l_5 as in *lat.*, ss_1 and ss_2 nearly as in *lat.*, ss_3 wanting, *as* as in *lat.*, ms_1 , ms_2 and ms_3 nearly as in *lat.* though a little smaller.

7g ♀: Ground slightly yellow-brown, l_1 begins squarely at the triangle, ends as in the foregoing specimen but the exterior black lines reach a little farther backwards, l_5 extends swelled alone to the wing-base, ss_1 and ss_2 as in *depr.*, ss_3 indicated especially on the one wing, *as* next to *lat.* but minor, ms_1 , ms_2 and ms_3 next to *lat.* but smaller.

8h ♀: Ground slightly yellow-brown, l_1 begins, pointed on the outer edge, at a short distance from the triangle, behind as in *lat.*, l_5 as in *lat.*, ss_1 and ss_2 nearly as in *depr.*, ss_3 wanting, *as* as in *lat.*, ms_1 and

ms_2 next to *lat.* but smaller, ms_3 next to *depr.* though a little larger.

9i ♀: Ground brownish-yellow, l_1 begins squarely near to the triangle, ends near to apex without a dilatation, l_5 and l_4 coalescent at the wing-base, ss_1 next to *depr.*, ss_2 as in *depr.*, ss_3 wanting, *as* as in *depr.*, ms_1 and ms_2 nearly as in *depr.*, ms_3 as in *depr.*

10j ♀: Ground yellow, l_1 begins squarely as in *lat.* at a distance from the triangle, continues as a line to the level of the ends of the other black lines and after that as a faint shadowing near to apex, ending as a triangular spot, l_5 and l_4 coalescent and enlarged on the one wing-base, on the other l_5 extending, swelled, alone to the base after a slight touch with l_4 , ss_1 and ss_2 as in *depr.*, ss_3 wanting, *as* next to *depr.*, ms_1 next to *lat.* but smaller, ms_2 as in *depr.*, ms_3 next to *depr.* though a little enlarged.

11 ♀: Ground yellow, l_1 beginning narrowed on the one wing, pointed on the other, at a short distance from the triangle, continues near to apex with a short interruption on the level of the other black lines and immediately thereafter triangularly dilated, l_5 coalescent with l_4 on the one wing, reaching on the other alone to the wing-base, ss_1 nearly as in *lat.*, ss_2 next to *depr.*, ss_3 wanting, *as* next to *depr.*, though a little larger, ms_1 , ms_2 and ms_3 next to *lat.*

12 ♀: Ground yellow, l_1 beginning squarely at the triangle, continues nearly to apex, ending with a faint dilatation, l_5 reaches, enlarged, alone to the wing-base, ss_1 nearly as in *lat.*, ss_2 next to *depr.*, ss_3 wanting, *as* next to *depr.*, ms_1 , ms_2 and ms_3 next to *lat.* but smaller.

13 ♀: Ground slightly brownish-yellow, l_1 beginning nearly square at a short distance from the triangle, ends as in *lat.*, l_5 extends enlarged alone to the wing-base, ss_1 enlarged, but next to *depr.*, ss_2 as in *depr.*, ss_3 indicated by a limited dissolution of l_2 to points on the place, *as* as in *lat.*, ms_1 next to *depr.*, ms_2 and ms_3 next to *lat.* but a little smaller.

14 ♀: Ground yellow, l_1 rounded off at the end, begins at a distance behind the faintly marked triangle, ends as in *lat.*, l_5 as in *lat.*, ss_1 and ss_2 next to *lat.* but smaller, ss_3 wanting, *as* as in *lat.*, ms_1 , ms_2 and ms_3 next to *lat.* but smaller. The specimen is monstrous with male fore-tarsi but with a female claw on the one fore-leg and for the rest being in want of claws on the fore- and middle-legs, partially, at least, from the pupal stage, evident from the pointed form of the last tarsal joint without any place for claws.

15 ♀: Ground yellow, l_1 begins square close to the triangle, ends near to apex as a row of points and without dilatation, l_5 extends after touching l_4 enlarged but alone to the wing-base, ss_1 and ss_2 next to *depr.*, ss_3 wanting, *as* triangular but smaller than in *lat.*, ms_1 next to *lat.*, ms_2 next to *depr.*, ms_3 next to *lat.*

16 ♀ (juvenis): Ground slightly yellow-brown, l_1 in front as in *depr.*, ends, narrowed on the one wing near to apex, on the other as a line like in *lat.* and after that only indicated by some faint points, l_5 as in *lat.*, ss_1 next to *lat.*, ss_2 next to *depr.*, ss_3 wanting, *as* next to *depr.* but enlarged, ms_1 , ms_2 and ms_3 next to *lat.* but smaller.

17 ♂: Ground slightly brownish-yellow, l_1 beginning squarely at a distance behind the triangle, ending as in *lat.*, l_5 as in *lat.*, ss_1 as in *depr.*, ss_2 as in *lat.*, ss_3 wanting, *as* as in *lat.*, ms_1 and ms_2 next to *lat.*, ms_3 on the one wing next to *depr.*, on the other next to *lat.*

18 ♀ (juvenis): Ground yellow, l_1 beginning squarely at a short distance from the triangle, ending as in *lat.*, l_5 reaches alone to the wing-base, narrowed on the one wing, on the other after a short interruption, ss_1 and ss_2 as in *lat.*, ss_3 wanting, *as* as in *lat.*, ms_1 , ms_2 and ms_3 ditto.

19 ♂: Ground slightly brownish-yellow, l_1 beginning squarely at a distance behind the triangle, ending as in *lat.*, l_5 reaches alone to the wing-base, on the one wing a little enlarged, ss_1 next to *lat.*, ss_2 as in *lat.*, ss_3 wanting, *as* as in *lat.*, ms_1 , ms_2 and ms_3 next to *lat.*, though smaller.

20 ♀ (juvenis): Ground slightly brownish-yellow: l_1 begins shortly behind the triangle, obliquely shortened at the exterior edge on the one wing, on the other sharpened to a point, continuing on the one wing near to apex without a dilatation, ending on the other as in *lat.*, having a point as the last rest near to apex, l_5 reaches, enlarged, alone to the wing-base, ss_1 and ss_2 next to *lat.* but smaller, ss_3 wanting, *as* next to *depr.*, ms_1 , ms_2 and ms_3 next to *lat.* but smaller.

21 ♂: Ground yellow-brown, l_1 beginning square near to the triangle, ending with a slight dilatation near to apex but interrupted on the level of the ends of the other black lines by a row of points, l_5 coalescent with l_3 and l_4 , reaches, much enlarged, to the wing-base, ss_1 and ss_2 as in *depr.*, ss_3 wanting, *as* next to *lat.* but minor, ms_1 , ms_2 and ms_3 next to *depr.*

22 ♂: Ground slightly brownish-yellow, l_1 beginning at a distance

behind the triangle with a short row of small points and ending as in *lat.*, l_5 as in *lat.* but with a short interruption in front on the one wing, ss_1 and ss_2 next to *lat.* though smaller, ss_3 wanting, as as in *lat.*, ms_1 , ms_2 and ms_3 next to *lat.* but a little smaller.

23 ♂: Ground slightly brownish-yellow, l_1 beginning rather squarely near to the triangle and ending without dilatation near to apex, l_5 extends, enlarged, alone to the wing-base, ss_1 next to *depr.* but a little larger, ss_2 next to *lat.* but smaller, ss_3 wanting, as next to *lat.* but smaller, ms_1 , ms_2 and ms_3 next to *lat.* but a little smaller.

In the same manner as before I have brought together these descriptions to the following Table 2.

TABLE 2. SUPPOSED *depressus* FROM DALARÖ. IN REALITY BASTARDS BETWEEN *depressus* AND *latescens* (BEETLES CAPTURES IN THE OPEN).
NUMBER 14 IS PARTLY MONSTROUS (VIDE THE DESCRIPTION)

Number	Ground colour	l_1 in front	l_1 behind	l_5	ss_1	ss_2	ss_3	as	ms_1	ms_2	ms_3
1a	n de	n de	new	new	n de	n la	= de	n. la	n. la	n. la	n de
2b	n de	new	. la	= la	= de	new	new	= la	n. la	n. de	n la
3c	n de	new	= de	n. la	n. de	n. de	= de	= de	n. la	n. de	n. de
4d	= la	new	n de	new	= de	n de	= de	n de	n de	n la	n la
5e	n. de	n la	= la	- la	n. de	n. la	= de	n. la	n la	n de	n. de
6f	= de	new	n la	- la	n la	n la	= de	= la	n la	n la	n. la
7g	n de	new	n la	new	= de	= de	new	n. la	n la	n la	n. la
8h	n. de	new	= la	= la	n de	n de	= de	= la	n. la	n. la	n de
9i	= de	new	new	= de	n de	= de	= de	= de	n. de	n. de	= de
10j	= la	= la	new	n de	= de	= de	= de	n de	n la	= de	n de
11	= la	new	new	new	n. la	n de	= de	n. de	n la	n la	n la
12	= la	new	n de	new	n lu	n de	= de	n de	n la	n la	n. la
13	n. de	n la	= la	new	n. de	= de	new	= la	n de	n lu	n la
14	= la	new	= la	= la	n la	n. la	= de	= la	n lu	n la	n la
15	= la	new	new	new	n de	n. de	= de	n la	n la	n de	n. la
16	n de	= de	new	= la	n. la	n. de	= de	n. de	n la	n la	n la
17	n de	= la	= la	= la	= de	= la	= de	= la	n. la	n. la	new
18	= la	n la	= la	n la	= la	= la	= de	= la	= la	= la	= la
19	n de	= la	= la	n. la	n la	= la	= de	= la	n. la	n la	n. la
20	n de	new	new	new	n. la	n la	= de	n de	n la	n. la	n. la
21	= de	new	new	= de	= de	= de	= de	n la	n. de	n. de	n. de
22	n. de	new	= la	n. la	n. la	n. la	= de	= la	n. la	n. la	n. la
23	n de	new	new	new	n. de	n. la	= de	n. la	n. la	n. la	n. la

It will I think be evident from Table 2 and from the descriptions that these 23 specimens taken directly from nature prove quite a similar fluctuation concerning the characters in question as my crossing-experiments have shown. For this reason there seems to be a firm ground for considering that hybridization had taken place in the open between the two mentioned species, yielding, of course, fertile offspring which have been able to maintain and to reproduce in extensive water such a strongly mingled population. The animals were very difficult to catch. During two months I was able, by means of *Juniper* branches, finally to collect only about 30 specimens, mostly adults. These may, of course, be considered as only a fractional part of the number of bastards living there. The three *juvenes* found prove that the population was still productive.

That in all bastards mentioned the interweaving of the characters in question, specific for *depressus*, resp. *latescens*, cannot be attributed to any increased variability of *latescens* because of its staying in brackish water, is proved by the simple fact that this species in the likewise brackish water of the bay at "Säby", very distant from "Dalarö", only occurs in its typical appearance, in so far I have been able to find out by my yearly draggings there. On the other hand that *latescens* in the quite fresh water of Mälär Lake at "Staket", exhibit an intermixture of *depressus*, I have already reported (FALKENSTRÖM 1932). A larva, captured there in 1930 in the 3d instar gave after hatching a male beetle which showed the following divergences from the type of *latescens*. Size larger and narrower (but not acuminate behind as *depressus*), the black lines on elytra coalescent, l_1 in front rounded and beginning at the sutural triangle, behind continuing near to apex, ending with a tooth-shaped dilatation, l_5 coalescent with the next two lines on each side to a broad band, reaching the wing-base. In all other respects the specimen is like a true *latescens*. It is the only specimen divergent from a typical *latescens* either captured on the place, or hatched at home from stocks, taken from this place.

In this connection I must mention that another small collection of *Deronectes* proves likewise a poor intermixture of *depressus* into *latescens*. This collection, now the property of the Entomological Museum of the Plant-Protection-Institute here, was made by Cand. J. SELLMAN in the neighbourhood of Västerås at Mälär Lake and was named by him *D. depressus*. F. The specimens agree, however, as regards all the

above detailed characters with the type of *latecsens* except three specimens which are only divergent with regard to the ending of l_1 at apex, since two of them in this character are like *depressus*, the third nearly so.

That intercrosses between *depressus* and *latescens* in the open are met with also outside Sweden, I had an opportunity to establish. In a fine collection of 65 specimens of *Deronectes*, most of them from Finland, which Magister HÅKAN LINDBERG of Helsingfors with his usual kindness had sent to me for inspection, there are together with true *latescens* doubtless many specimens which exhibit more or less intermixture of *depressus*. Exclusively typical *latescens* are from Finnström, Saltvik, Hammarland, Jomala and Sund. Together with typical *latescens* specimens with a poor intermixture of *depressus* are from the following localities: Ivalojoiki: 1 ex., Uusikaupunki: 3 ex., Tvärminne: 3 ex., Sortavala: 8 ex. and Ekerö: 5 ex. Specimens with a little more of *depressus* are from Kittilä: 1 ex., Kemi: 1 ex., and Lojo: 1 ex. A very high intermixture of *depressus* prove all specimens 6 ex., marked from Sordavala with form of *depr.*, ground colour of *lat.*, strongly coalescent lines on elytra of a peculiar shining black colour, as laid on superficially, yellow spots of mixed composition, male fore-claws sometimes like *lat.*, sometimes like *depr.*, on the whole, thus, clear bastards between the mentioned species. Although all these specimens had been sent to me as *depressus*, there were only two females from Suomussalmi which come very near to this species. On account of their very different size which moreover is too small for *depressus* and because of some other details I do not believe that they belong to this species but are bastards.

Among the specimens from Ekerö there was one, not considered before, which is rather like a *D. griseostriatus* DE G. This species, however, has wings without a tooth towards the apex, while the specimen in question has such a one on each wing like *latescens*, *depressus* and others. For that reason I have, as mentioned in a previous paper (FALKENSTRÖM 1932) suggested an intercross between *griseostriatus* and *latescens* or *depressus*.

b. Larval stage

1. Pure species, mainly

If anyone should find reason to object to this report on my crossing-experiments between *depressus* and *latescens* on the grounds that the number of reard bastards is too small for a decisive opinion, which I can in no wise admit, I am prepared to increase my argumentation by means of ontogeny. As pointed out above, I was forced to reduce the number of larvae in the respective series in order to get time for the necessary labour of individual control of the offspring. The control was performed thus. The eggs from the four crossed pairs were transferred to glass-cases, one for each pair. These cases were often inspected, and every hatched larva was transferred to its own case. Under my daily inspection (twice a day at least) and distribution of the food, the larvae had to accomplish their whole metamorphosis into the cases which I supplied. The pupal stage was passed through likewise during complete isolation of every individual. After hatching every beetle obtained its own glass-jar, where it spent its time, isolated to its death, unless it was required for reproduction. All receptacles were, of course, seriated and numbered. During the metamorphosis the different exuviae of each larva and its pupa were gathered and put into a glass-tube, marked with the number and series of the specimen in question, by means of which I can, when necessary, review the earlier stages of a specimen in regard to many details. By the restriction of the larval series it was the earlier stages which were subjected to elimination. Although I certainly have sufficient material for my argumentation, I think it better, for the present at least, to restrain a little. For that purpose I will deal chiefly with the third larval stage and some of its colour details which are accessible without difficulty for comparison. First I should like to lay stress upon some moments in the appearance of the larvae in all three stages and refer for the rest to my paper (FALKENSTRÖM 1933) quoted above.

In *depressus* 1st stage, nearly the whole upper side and especially the dorsal plates of the 2d–6th abdominal segments are dark (grayish-black to brownish-black). In *latescens*, 1st stage, the upper side is considerably paler with the 3d–6th abdominal segments white and

the other segments to a greater or less extent grayish-black. This larva is just as broadly barred in black and white, as *depressus* is rather monotonously dark above. One can, thus, at once discriminate between these two kinds of larvae. I have examined more than 1500 specimens of them during the past years and never found any divergence in this regard, irrespective of the way in which the progeny of the true species were produced, i.e. by interbreeding in my stocks, by breeding of fresh material from nature or by capture of larvae in the open.

The 2d stage of *depressus* exhibits a very different colouring in comparison with the 1st stage of the same species, viz. the upper side shows on a slightly yellowish white ground a distinct black colour pattern giving to the larva a broadly barred appearance in black and white. The 2d stage of *lutescens* is nearly a magnified copy of its 1st stage, because of which this larva becomes almost like that of *depressus* in the same stage, but with the dark colour more faint and in some details different from that of *depressus*.

The 3d stage of *depressus* is as regards the colour pattern on the whole a magnified copy of its 2d stage with a saturated black colouring on a deep yellow (butter or egg) ground, here too giving to the larva a broadly barred appearance in black and yellow. The 3d stage of *lutescens* is to a very essential degree different from *depressus* in the same stage, since the whole upper side is speckled in brown and yellow in such a manner that the main colour is brown on a pale lemon-yellow ground, with the ground-colour coming insight as distinctly located, large and small, yellow spots in the brown colour. In this larva the barred design has been entirely removed.

Some of the details now pointed out are easy to recognize in the photos and drawings in my papers (FALKENSTRÖM 1932 and 1933), where unfortunately some other details are more indistinct on account of the translucent, dark contents of the intestines, respectively of the reflections when photographing the living animals in water.

There are some other details in the larval colouring which I may further lay stress upon. With reference to Plate II, figg. 1 and 2 of this report and to the figures in my papers quoted just above we see in *depressus*, the 3rd instar, that from the black occiput and collum on each side of the faint line (suture) in the middle, a black, outwards bent stripe passes to the base of the antenna. The two stripes make a

figure which I have called the front-fork. This has, as shown, solid arms, tapering forwards, following and confining the frontal sutures. Much the same figure is to be seen on a larva of the 2d stage of this species. The 3d instar of *latescens* has also a front-fork, but the fork-arms are at the base divided into an interior and an exterior branch which soon join outwards, forming an oval hole at the base, a loop-hole where the yellow ground-colour is translucent, for reason of which I have called such a front-fork looped. Also in the 2d stages of *latescens* the front-fork is looped, while in the 2d stage of *depressus* the front-fork has solid arms like its 3d stage. This very striking different formation of the front-fork in these two species is thoroughly constant.

One ought also to mention that in the 2d stage of *depressus* the solid arms of the front-fork pass outside, in the 3d stage, as said, on each side of the frontal suture, thus closely confining the same. In the 2d and 3d stages of *latescens* the looped fork-arms let their interior branch pass inside, the exterior outside the frontal suture (because of which the denomination) but at a little distance from the sutures. As these together with the median sutures of vertex and the pectoral segments denote the places where the larval skin bursts at the ecdysis, it is evident that the differences in question between the two species may be considered as very important ones from a constitutional point of view. This is strengthened by the fact that in the 3d stage of *depressus* the fork-arms are solid, though they confine, as mentioned, the frontal sutures. Which fact shows on the other hand that the sutures do not constitute the primary reason for looped fork-arms in *latescens*.

As in treating of the imaginal stage, the differences in ground-colour and colour pattern were but a small part of all differences proved in *depressus* and *latescens*, the same is the case with regard to the differences above noted between their larvae which may easily be gathered from the descriptions in my paper quoted (FALKENSTRÖM 1933). When treating of the bastards between the two species in the 3d stage, I shall restrict myself to the ground-colour, the colour and distribution of the dark pattern on the upper side and the form of the frontal fork. I shall include for comparison the larvae of the hatched beetles, described above. The following may be well marked. As a loop-hole is said to be wanting on the arm of the frontal fork, this is

not to be understood as if the base of the fork-arm were as broad as in *depressus*. On the contrary, the base is then narrower, i.e. it is simple, since the interior branch is wholly or in its posterior portion wanting. When treating of the bastards between *depressus* and *elegans* in another paper, I shall show a very remarkable difference between the two kinds of bastards as regards this character, by means of which one can discriminate them.

2. Larval bastards, 3d instar. F₁-generation

As the series are the same to which the hatched beetles, detailed above, belong, the series A and C will indicate intercrossing between *depressus* ♂ and *latescens* ♀; series B and D between *latescens* ♂ and *depressus* ♀. In Table 3, after the descriptions, those larvae which were reared to beetles are marked by an asterisk.

Series A: *depressus* ♂ × *latescens* ♀.

A₁: Ground yellow, pattern grayish-brown, disposed nearly as in *lat.*, fork-arms simple, i.e. with narrow base.

A₂: Gr. lemon-yellow, patt. yellow-brown, disp. = *lat.*, fork-arms simple.

A₃: Gr. lemon-yellow, patt. yellow-brown, disp = *depr.*, fork-arms simple.

A₅: Gr. lemon-yellow, patt. grayish-brown disp. = *lat.*, fork-arms simple.

A₆: Gr. yellow, patt. grayish-brown, disp. n. = *lat.*, fork-arms simple.

A₇: Gr. wax-coloured, patt. grayish-brown, disp. n. = *lat.*, fork-arms simple, but with an interruption.

A₈: Gr. lemon-yellow, patt. yellow-brown, disp. = *lat.*, fork-arms simple, but with an anterior rest of the inner branch.

A₉: Gr. yellow, patt. grayish-brown, dispos. n. = *lat.*, fork-arms simple.

A₁₀: Gr. lemon-yellow, patt. yellow-brown, disp. = *lat.*, fork-arms simple.

A₁₁: Gr. lemon-yellow, patt. yellow-brown, disp. = *lat.*, fork-arms simple.

A₁₂: Gr. lemon-yellow, patt. yellow-brown, disp. n. = *lat.*, fork-arms simple.

A13: Gr. lemon-yellow, patt. yellow-brown, disp. n. = *lat.*, fork-arms simple.

A14: Gr. lemon-yellow, patt. yellow-brown, disp. = *lat.*, fork-arms simple.

Series C: *depressus* ♂ × *latescens* ♀.

C1: Gr. wax-colour., patt. grayish-brown, disp. n. = *lat.*, but with larger yellow spots on the abdominal segments, fork-arms simple but with an anterior rest of the inner branch.

C2: Gr. lemon-yellow, patt. yellow-brown, disp. n. = *lat.* but with more yellow colour translucent on the abd.-segm.s, fork-arms simple.

C3: Gr. lemon-yellow, patt. yellow-brown, disp. = *lat.*, fork-arms simple.

C4: Gr. yellow, patt. grayish-brown, dispos. n. = *lat.* but with larger yellow spots on the abdom.-segm.s, fork-arms simple.

C5: Gr. lemon-yellow, patt. yellow-brown, disp. = *lat.*, fork-arms simple but with an anterior rest of the inner branch.

C6: Gr. lemon-yellow, patt. yellow-brown, disp. = *lat.*, fork-arms simple but with an anterior rest of the inner branch.

C7: Gr. lemon-yellow, patt. grayish-brown, disp. n. = *lat.*, fork-arms simple.

C8: Gr. yellow, patt. yellow-brown, disp. = *depr.* with the 2d-4th abdom.-segm.s, yellow, fork-arms simple but with a short interruption near the base.

C9: Gr. lemon-yellow, patt. yellow-brown, disp. = *depr.* with the 2d-3d abd. s.s yellow, fork-arms simple.

C10: Gr. lemon-yellow, patt. yellow-brown, disp. = *lat.*, fork-arms simple but with an anterior rest of the inner branch.

C11: Gr. lemon-yellow, patt. yellow-brown, disp. = *lat.*, fork-arms simple.

C12: Gr. lemon-yellow, patt. grayish-black, disp. n. = *lat.*, fork-arms simple.

C13: Gr. lemon-yellow, patt. yellow-brown, disp. n. = *lat.*, fork-arms simple but with an anterior rest of the inner branch.

C14: Gr. yellow, pattern and its disposition forming a composition of both, types, fork-arms simple but with a faint anterior rest of the inner branch.

Series B: *latescens* ♂ × *depressus* ♀.

B1: Gr. creamy, patt. light yellow-brown, disp. n. = *lat.*, fork-arms simple.

B2: Gr. creamy, patt. grayish-brown, disp. = *lat.*, fork-arms simple but with an anterior rest of the inner branch.

B3: Gr. yellow, patt. grayish-brown, disp. = *lat.*, fork-arms simple but with an anterior rest of the inner branch.

B4: Gr. yellow, patt. grayish-black, disp. n. = *depr.*, fork-arms with loop-hole.

B5: Gr. yellow, patt. dark grayish-brown in front, disp. n. = *depr.*, fork-arms with loop-hole.

B6: Gr. yellow, patt. grayish-brown, disp. = *depr.*, fork-arms with loop-hole.

B7: Gr. yellow, patt. grayish-black, disp. n. = *depr.*, fork-arms simple but with an anterior rest of the inner branch.

B8: Gr. yellow, patt. grayish-black, disp. n. = *lat.*, fork-arms simple but with an anterior rest of the inner branch inside the eye.

B9: Gr. yellow, patt. grayish-brown, disp. n. = *lat.* but with a large, yellow spot on each side of the 2d-3d abdom.-segm.s, fork-arms simple.

B10: Gr. yellow, patt. yellow-brown, darker in front, disp. = *depr.*, fork-arms simple.

B11: Gr. yellow, patt. grayish-brown, disp. n. = *depr.*, fork-arms with loop-hole.

B12: Gr. yellow, patt. grayish-brown, disp. = *lat.*, fork-arms simple.

B13: Gr. lemon-yellow, patt. yellow-brown, disp. = *lat.*, fork-arms simple but with an anterior rest of the inner branch.

B14: Gr. lemon-yellow, patt. grayish-brown, disp. = *depr.*, fork-arms simple.

B15: Gr. yellow, patt. grayish-black, disp. = *depr.*, fork-arms with loop-hole.

B16: Gr. yellow, patt. grayish-brown, disp. = *depr.*, fork-arms with loop-hole.

B17: Gr. yellow, patt. grayish-black, disp. = *depr.*, fork-arms simple.

B18: Gr. yellow, patt. grayish-black, disp. = *depr.*, fork-arms with loop-hole.

B19: Gr. yellow, patt. dark grayish-brown, disp. as a composition of both types, fork-arms simple.

B20: Gr. grayish-white, patt. pale grayish-brown, disp. n. = *lat.* but with much pale intermixture, fork-arms simple but with a rest of the inner branch.

B21: Gr. yellow, patt. black, disp. = *depr.*, fork-arms with loop-hole.

B22: Exhibits thoroughly strange appearance, quite different from any of the parent-types, except head and pronotum. The remaining part of the upper side is uniformly grayish-black with a small light spot on the side of each segment and with the 7th abdominal segment white at the apex, fork-arms simple.

B23: Gr. yellow, patt. yellow-brown, disp. = *depr.*, fork-arms with loop-hole.

B24: Gr. yellow, patt. grayish-brown, disp. n. = *lat.* but with very extended light intermixture on the segments, fork-arms simple.

B25: Gr. grayish-white, patt. grayish-brown, disp. n. = *depr.*, fork-arm simple.

B26: Gr. yellow, patt. grayish-brown, disp. = *depr.*, fork-arms with loop-hole.

B27: Gr. grayish-white, patt. pale grayish-brown, disp. n. = *lat.* but with very much of the pale ground visible on the segments, fork-arms with loop-hole.

B28: Gr. yellow, patt. black, disp. = *depr.*, fork-arms with loop-hole.

B29: Gr. grayish-white, patt. grayish-black, disp. unlike any of the parent types with the dark colour rather uniformly distributed over the whole upper side, except head, pronotum and the 2d abdominal segment, fork-arms with loop-hole.

B30: Gr. yellow, patt. grayish-black, disp. n. = *depr.* fork-arms with loop-hole.

B32: Gr. lemon-yellow, patt. yellow-brown, disp. = *depr.*, fork-arms simple.

B33: Gr. yellow, patt. grayish-black, disp. = *depr.*, fork-arms simple.

Series D: *lutescens* ♂ × *depressus* ♀.

D1: Gr. creamy, patt. grayish-brown, disp. n. = *depr.*, fork-arms with loop-hole.

D2: Gr. pale yellow, patt. grayish brown, disp. n. *lat.* but with darker meso-, metanotum and 1st abdom. segm. (= *depr.*), fork-arms with loop-hole.

D3: is like D2.

D4: is like D2.

D5: is like D2.

D6: Gr. yellow, patt. grayish-brown, disp. n. = *lat.*, except that the last two body-segments and the 1st abdom. segm. are darker grayish-brown, fork-arms with loop-hole.

D7: Gr. yellow, patt. grayish-black, disp. = *depr.*, fork-arms with loop-hole.

D8: Gr. lemon-yellow, patt. grayish-black, disp. = *depr.*, fork-arms with loop-hole.

D9: Gr. yellow, patt. black, disp. = *depr.*, fork-arms with loop-hole.

D10: Gr. lemon-yellow, patt. grayish-black, disp. n. = *lat.* but with more of yellow spots visible, fork-arms with loop-hole.

D11: Gr. yellow, patt. grayish-brown, disp. n. = *depr.*, fork-arms with loop-hole.

D12: Gr. lemon-yellow, patt. grayish-black, disp. = *depr.*, fork-arms with loop-hole.

D13: Gr. yellow, patt. grayish-black, disp. n. = *depr.*, fork-arms with loop-hole.

D14: Gr. yellow, patt. grayish-brown, disp. n. = *depr.*, fork-arms with loop-hole.

D15: Gr. yellow-white, patt. grayish-black, disp. = *depr.*, fork-arms with loop-hole.

D17: Gr. yellow, patt. grayish-brown, disp. n. = *depr.*, fork-arms with loop-hole.

D18: Gr. yellow, patt. grayish-brown, disp. n. = *depr.*, fork-arms with loop-hole.

D19: Gr. yellow, patt. black, disp. = *depr.*, fork-arms with loop-hole.

TABLE 3. BASTARD-LARVAE OF *Deronectes depressus* AND *D. latescens*
 3D INSTAR, IN CULTURE DURING 1931
 (An asterisk denotes larvae whose imagines are detailed above.
 Abbreviations as before, or else readily understood).

Series and number in the series	Ground colour	Colour of the pattern	Disposition of the pattern	Fork-arms with loop-hole	Fork-arms with a rest of the inner branch	Fork-arms simple, seldom with an interruption
A1*	=dc	new	n. = la			yes
A2*	= la	= la	= la			yes
A3	= la	= la	= dc			yes
A5	= la	new	= la			yes
A6*	dc	new	n. = la			yes
A7*	new	new	n. la			interrupt
A8	= la	= la	la		yes	
A9*	= dc	new	n. = la			yes
A10*	= la	= la	= la			yes
A11	= la	= la	= la			yes
A12	= la	= la	n. = la			yes
A13	la	= la	n. = la			yes
A14	= la	= la	= la			yes
C1*	new	new	new		yes	
C2*	= la	= la	new			yes
C3	= la	= la	= la			yes
C4*	= dc	new	new			yes
C5*	= la	= la	= la		yes	
C6	= la	= la	la		yes	
C7*	= la	new	n. = la			yes
C8	= dc	= la	= dc			interrupt.
C9	= la	= la	= dc			yes
C10	= la	= la	= la		yes	
C11	= la	= la	= la			yes
C12	= la	n. de	n. la			yes
C13	= la	= la	n. la		yes	
C14	= de	new	new		yes	

Series and number in the series	Ground colour	Colour of the pattern	Disposition of the pattern	Fork-arms with loop-hole	Fork-arms with a rest of the inner branch	Fork-arms simple, seldom with an interruption
B1*	new	new	n.la			yes
B2*	new	new	=la		yes	
B3*	=de	new	=la		yes	
B4*	=de	n.de	n.de	yes		
B5*	=de	new	n.de	yes		
B6	=de	new	=de	yes		
B7	=de	n.de	n.de		yes	
B8	=de	n.de	n.la		yes	
B9*	=de	new	new			yes
B10	=de	n.la	=de			yes
B11	=de	new	n.de	yes		
B12	=de	new	=la			yes
B13	=la	la	=la		yes	
B14	=la	new	=de			yes
B15	=de	n.de	=de	yes		
B16	=de	new	=de	yes		
B17	=de	n.de	=de			yes
B18	=de	n.de	=de	yes		
B19	=de	new	new			yes
B20	new	new	new		yes	
B21	=de	=de	=de	yes		
B22	new	new	new			yes
B23	=de	=la	=de	yes		
B24	=de	new	new			yes
B25	new	new	n.de			yes
B26	=de	new	=de	yes		
B27	new	new	new	yes		
B28	=de	=de	=de	yes		
B29	new	n.de	new	yes		
B30	=de	n.de	n.de	yes		
B32	=la	=la	=de			yes

Series and number in the series	Ground colour	Colour of the pattern	Disposition of the pattern	Fork-arms with loop-hole	Fork-arms with a rest of the inner branch	Fork-arms simple, seldom with an interruption
B33	--dc	n de	--de			yes
D1*	new	new	n.de	yes		
D2*	new	new	new	yes		
D3*	new	new	new	yes		
D4*	new	new	new	yes		
D5*	new	new	new	yes		
D6*	-dc	new	new	yes		
D7	--de	n.de	--de	yes		
D8	--la	n d.	--de	yes		
D9	de	de	--de	yes		
D10	-la	n de	new	yes		
D11	de	new	n de	yes		
D12	-la	n.de	- de	yes		
D13	de	n de	n.de	yes		
D14	- de	new	n de	yes		
D15	new	n.de	= de	yes		
D17	--de	new	n.de	yes		
D18	--de	new	n.de	yes		
D19	--de	-- de	= de	yes		

From the descriptions above and from Table 3 of bastard larvae of *depressus* and *lutescens*, it is evident that even here, with one exception, an irregular variation of the characters examined appears, and that the parental individuals had different capacities to let the specific characters of their own species reappear in the offspring. The exception alluded to is, that in the D series looped front-forks occur exclusively. This apparent dominance of the specific character of *lutescens*, pure race, is, however, balanced by the variation of the same character in the parallel B series. As all specimens of *depressus* used in the four series are brother and sisters born at my home in 1930 and minutely examined by me during their whole metamorphosis without any remark in the journals as regards their solid front-forks, a

character which caught my eyes at once by comparison with my larvae of *latescens*, taken in the open during the same year, the presence of looped fork-arms may probably be considered to be due to the male used in the D series. In this case it is noteworthy that in the parallel B series not even half of the bastards showed looped fork-arms. There is the difference between the two males of *latescens* used in these two series that the male of the B series is from "Säbyviken" (a bay of the Baltic Sea) caught as larva in the 2d stage, while the male of the D series is from "Stäket" (on Malar Lake). caught as larva in the 3d stage, both in the same year, viz. 1930. The former is from brackish, the latter from fresh water. That is the sole real difference between both males apart from the fact that one larva, captured together with the male of the D series as larva, proved not to be of pure race but with a slight intermixture of characters, specific for *depressus* as mentioned above. As looped front-forks do not occur in the latter species and the males of the B and D series showed the typical appearance of *latescens*, it is evident that the power of heredity as regards the characters in question is *individually* different. Of the hatched beetles in both series there are in the B series 2 ♂ with simple, 1 ♂ with looped fork-arms, 1 ♀ with ditto and 2 ♀ with small vestiges thereof (rests of the inner branches), but in the D series 4 ♂ and 2 ♀, all with looped fork-arms. This character is, thus, not linked to sex. How the difference in question is to be interpreted, I am at present not able to decide.

A continued account of the appearance of the bastards in the earlier stages, even if restricted to colour differences, would certainly multiply the series very considerably, but hardly to any benefit proportionate to the labour bestowed thereon. For that reason I shall only mention the following. While in the pure species in question, the larvae of the 1st stage are distinctly and uniformly divergent from each others, *depressus* having its dorsum on the whole rather dark (brown), *latescens*, on the contrary, white (*vide* FALKENSTRÖM 1933), the bastards have a varying number of the dorsal abdominal plates, counted from the front, dark but always some apical ones white.

From the crossing-experiments I got, as shown, an elucidation of the intermixture of the otherwise specific colour characters in both species in question, which had appeared in the population of the supposed *depressus* at Dalarö. With that I was so far sufficiently

satisfied. As in the dry spring of 1932 the food-supplies of red *Chironomus*-larvae decreased swiftly, I was forced to kill the bastards in order to sustain my stocks of pure species. It might otherwise have been of interest to be informed of the 2d filial generation. With regard to the easily performed intercrosses of the parents in both directions, to the normal progress of the embryonal and larval development without increased mortality, to the evident vitality of the bastards during their whole metamorphosis and, above all, to the presence of bastards, old and juvenes, in such an extended water as in the bay at Dalarö, I was, however, not in doubt concerning the fertility of the experimentally produced bastards.

As already mentioned several times, even the colour characters, used above for the estimation of the bastards from the open and those of my crossing-experiments as well, have always shown so high a degree of constancy in the pure species that these are readily and distinctly discerned from each others. As these waterbeetles in captivity must carry on their life in a manner so essentially different in every respect from that in the open, one might have expected that, during the metamorphosis with its repeated, sensible periods, signs of divergences from individuals captured in the open should have appeared. But not the faintest trace thereof has been noticed. Especially the conditions which I have been able to give to their pupal stage seem to offer hazardous moments. Under nature the larvae, in order to pupate, bury themselves in the earth in places protected against drought, at a short distance from the water-edge. At my home the larvae entered into the earth, in the beginning wet, but gradually drying up. As the pupating usually takes place during the height of summer as in the open, it often happens that the earth in the dry and uniformly warm room air dries to a powder. Nothing like that occurs, I am sure, under natural conditions, where, apart from rain, the night-moisture as well as the subsoil water preclude such a dryness. Of course, even indoors, this could be prevented by special arrangements. As I, however, long ago found that permanent humidity in the soil indoors entailed still greater peril for the pupa in the form of parasitic attacks (fungi, nematods, mites, dipterous larvae etc.) I let the matter rest. Moreover, the uniform temperature indoors is about 5–10°C higher than that of the soil where the pupae live in the open. Due to this fact the pupal stage is passed a little more

quickly than in the open. Though these two circumstances together, especially if they are accidentally intensified, can lead to deformities, for instance shortened or wrinkled wing-cases, I have never seen any irregularity as regards colour pattern and other characteristics result therefrom.

c. Colour characters -- hereditary

Just as the larvae after ecdysis appear colourless or white, so the beetles emerge from the pupal exuviae white except the black eyes and the yellowish-brown mandibulae and claws. It takes about one day for the hatched beetle to get its colour pattern, but weeks until the chitinous integument grows hard and gets its definitive ground colour. It is, thus, after hatching that the beetle gets its colour and colour pattern, but it is still in darkness, as the beetles rest in their pupal holes for some days after ecdysis. The anatomic-histologic predispositions to the colouring are, however, made already during the pupal stage. According to the investigations of TOWER (1903) as regards the colours of insects, spots and lines in the beetles are as a rule situated in the primary, homogenous and rather thin cuticula. Under this lies the secondary one, stratified and gradually growing thicker, which, like the primary, has the underlying hypodermis as origin. On microscopic sections one can see the secondary cuticula perforated by small pore-canal, beginning in hypodermis and ending at the primary cuticula, but not perforating this. On more or less limited places in the hypodermis a zymogen is generated which, passing through the pore-canal, acts as an enzyme on the primary cuticula and causes a chemical transmutation in the latter. This was more fully advanced by TOWER (l.c.). The intensity of the colour depends, of course, upon the quantity of acting enzyme, but the appearance of the colour and colour pattern is exclusively connected with the hardening of the chitinous integument according to TOWER (l.c.). Besides this colouring, long ago known as the chemical or pigment colouring, there is in insects and even in other animals a physical one, due to a certain structure of the body surface as fine punctures, striations, thin, differently refractive membranes etc. Most common is a combination of pigment and structure colouring. In the phytophagous beetles, for instance *Leptinotarsa*, the ground

colour is, according to TOWER (l.c.) partially hypodermatic on account of the percentage of chlorophyll in the food.

There can certainly not be any difference of opinion regarding the fact that a more or less extensive body structure of the beetles producing colour like every other morphological formation of the same, may be considered as founded on their genetic constitution and, thus, hereditary. There is as little ground to deny the same hereditary nature in the anatomical and histological arrangements for chemical reactions in the primary cuticula which produce colour and colour pattern in the beetles. As a systematist I have myself never doubted that the colour and colour patterns, described above in the different stages of the two species of *Deronectes*, were founded on their constitution and thus hereditary. And I am convinced that every other systematist with experience in coloured animals holds a similar opinion as regards his groups of animals. On account of this, it was very surprising to see, in almost all genetic texts and handbooks, the colour pattern in *Leptinotarsa* according to TOWER's own account (TOWER 1906) pointed out as instances among animals of fluctuating characteristics, caused by the environment, i.e. as modifications or somatic formations. I willingly admit the difficulty of getting a firm hold of TOWER's real opinion in regard to these questions in his extensive work. For he says in some places that the distinct formation of the colour pattern in the different species of *Leptinotarsa* (without connecting forms between the species) depends upon the germ plasma of the gametes; in other places that the variations during his experiments are of somatic nature. As to the latter I shall only cite what TOWER (l.c. 1906, page 261 below) wrote as to his plate 25 as regards *decimlineata* so commonly quoted in the genetic works: "The entire negative results in this experiment may be interpreted as an example of the total inefficiency of selection, or, what is more probable, as indicating, that the selected variations of the character were not capable of transmission, being pure fluctuating somatic variations. In the chapter on coloration I have shown, that color variations produced by environmental conditions during the larval and pupal periods are not inherited at all."

The meaning of TOWER's experiments, begun long before 1906, with the genus *Leptinotarsa*, the different species of which are so highly variable in their colour characters, was, according to his own

words (l.c.), to verify whether one could, by isolation and continued culture of individuals showing extremities in certain colour characters simple or complex, produce all the intermediates to the next similar species, or just verifications of DARWIN's hypothesis that, by selection of the present small divergences from the species norm, a species alters into another nearly related one. The failure of his experiments in this respect seems to have been such a severe disappointment that TOWER yielded to the biometricians, though already at that time, according to his own words, he well knew that their working methods were not suited to a distinct discrimination between hereditary variations and such variations caused by environment.

From his similarly extensive work published 12 year later it is not difficult to see that TOWER realizes he has made a mistake in his work of 1906 though he tries to conceal it, throwing the blame on Biometrics. In his last work (TOWER 1918) he writes pages 324-25 following in this respect very suggestive words: "The area of the pigment exposed on the pronota of my material may well be equal in area or proportion of surface exposed, but the pattern conditions presented, the array of active agents, factors, and determiners present and that are brought into this array, give so extensive opportunity for heterogeneous conditions that the real nature of the series is constantly shifting and is hopelessly confused, so that the result statistically is to end with the "conclusion" that the "variations" are somatic and so not inheritable. They may be so or not; the method has not been able to test the point. I have seen series in which the conditions present were known to be accurately inherited and recognizable by the arrangements in the pattern, but the biometric method resulted in a hopeless array of ranging areas of pigment, which could not be fixed. It is this recognition of the results of the confusion of real conditions under the blanket of biometrics that formed the transition from the former situation to the present one. The results attained in this first set are correct as far as they can be with the method employed, and were carried as far as it was possible to go in nature and in the experimental analysis of the problem of place variation.

As has been shown in the preceding pages, this pattern is not a haphazard array of colored areas, but an accurately constructed system, in which the color is the end-result of many agents, and from this

point of view quite different conditions are conceived of in the population as the cause of the heterogeneity found, and so the phenomena of place and geographic variation take on a new aspect, becoming capable of analysis along new and profitable lines. This is not the place to deal with the gametic constitution of the material used or of the factors that have been recognized as present and acting in the population in this complex system."

That TOWER through his continued experiments became convinced that his polygons of variation from 1906 were anything but somatically founded is less surprising than that the genetic authors after 1918 continue to quote passages from TOWER's work of 1906 no longer approved by himself. TOWER's two works contain much of interest and importance for the experimenting scientist, but in this respect he is not quoted but rather discredited. At the time of TOWER's experiments for his work of 1906 those questions were still of highest interest which he wanted to have answered in an objective way in the nearest contact with nature. During half a century they held the centre of an intensive theoretical discussion all over the world. The results of his experiments reported in his work, are, in spite of his miss-interpretation in some respects, nevertheless of the greatest importance for the doctrine of evolution. They may come into their own, even though TOWER happened to advance them at a moment when the "re-discovery" of MENDEL's experiences gave another direction to the studies.

Though TOWER failed to support by his experiments DARWIN's postulate that a species by selection and preserving of the small variations could be connected with another related one, so are neither his own conclusions nor those of the geneticists quoting him that the variations of the colour characters observed should be somatic, in no wise logical consequences of this failure. Rather may it be so that TOWER's incapacity to alter the colour characters from the specific norm beyond a certain degree indicates their constitutional nature. How could this be better proved than in TOWER's experiments under different environmental conditions with populations of the different species? That displacements of the characters in question, although small, as he says, could take place, is by no means a motive for considering either the colour characters as such, or the variations observed under the altered conditions as being of somatic nature.

These variations can all the same be responses of the genes, regulating the colouring, to the experimental conditions of environment and certainly they are so. TOWER says himself (l.c. 1906), summing up the results of his experiments under different conditions of environment, that the latter do not prove any effect specific for them but that they act only as "stimuli". It may thus be obvious to everybody that TOWER with his experiments has manifested the colour and colour pattern of his material as being founded in and regulated by the individuals' constitution, thus, the very contrary to his own and his followers' conclusions.

I have in my cited paper (1935) nearer stated the reasons for my opinion that the action of the genes and that of the environment neither can, nor may be separated from each other. As long as Genetics holds the primary, more abstract standpoint, conceiving the norm of reaction as the specific in the races and, thus, the different mode of reaction against any agents as the real ground to their proved differences, it was not much further to the consideration of an altered condition as alone responsible for an eventually appearing effect. But if one accepts, as nowadays usually and I think for good reasons the gene-theory and MORGAN's principle of the distinct location of the genes in the chromosomes, one has also accepted the occurrence in the cells of the organisms individualized, living and specifically acting invisible particles. These are according to the theory responsible for heredity, that is to warrant the identity of the offspring with the parents. This task can only be carried out, if the genes are responsible for the life in the cells at least during the periods of development and cellular regeneration. As living things the genes are susceptible to influences. But since man is not able to govern the cellular reactions, it is irrelevant to speak of exclusive environmental action. Environment can only act as a stimulans, for the effect the genes are alone responsible, each according to its specific nature. In consequence thereof I distinguish strongly between the environment of the individual and that of the genes. Only the latter, i.e. the cell-contents, has importance for the reactions of the

genes under common, natural conditions. From MORGAN's principle of distinct location of the genes their solid consistence follows. But as "corpora non agunt solida" it follows also that they must act by specific enzymes or other secreted matters.

In this connection I will lay stress upon the fact that genes and genetic factors are not congruent conceptions. The latter are the most extensive in regard to their contents and many such factors are pure environmental conditions acting as stimuli. This is self-evident for everybody who has learned to discern between the environment of the genes and that of the individual which bears them, and who comprehends the manifestation of life in the reproductive cells as decisive for the individual.

According to my conceptions just given, it seems to me that the importance of the fluctuating variations emphasized in the genetical textbooks as criterion of exclusive environmental effects rests upon a false ground and stands in urgent necessity of revision. Much might be added, but I will only hold forth that several examples of fluctuating variation are chosen without due critic. As for instance the often quoted series of leaves of a plum-tree (after DE VRIES). It seems to me that one could as well place in a row stones gathered on the shore of a lake as example of the result which a complex of many different, uncontrolled acting powers can give. Anything instructive for Biology can not be attained in that manner.

Having with these comments attempted to remove eventual doubt about the constitutional nature of the colour and colour pattern in the two species of *Deronectes* which I examined and intercrossed I will pass on to speak of the species *latescens* described by me as worthy of consideration as a true species.

d. Deronectes latescens — a true species

As the purpose of this paper is in the main to report on species-crossing in animals and the conditions for that, it is of importance that no doubt as to the specific rank of *latescens* may occur. As known there are no rules which determine when a discovered form may be entitled to a specific name. It is the author's task to propose and his scientific colleagues' right to accept, or to refuse. For about 20 years I have made extensive studies of *Dytiscidae* and some other families

of water-beetles and described many new species and cleared up several wrongly described ones without disapprobation. According to my statements given above, not only the imaginal stage of the individual of my species but also all earlier stages, partly in a very striking manner, show plenty of distinct divergences from corresponding characters in the only species which can be thought of otherwise, viz. *depressus*. Moreover, I have by means of careful investigations proved that the appearance of some individuals, captured in the open and showing intermixture of colour characters otherwise specific for each of the two species in question, can be produced by intercrossing of their representatives. On account of that I cannot withdraw my opinion that few if any wild animal species are more entitled to be considered a true species than *latescens*. By a specialist this species can easily and without hesitation be separated from *depressus* on account of the characters given in my description of 1932 (l.c.). Since that time I have seen many specimens of *latescens* from other places in Sweden and from abroad. Only in one case I have found a slight divergence from my original description, when individuals from western Sweden (Värmland) kindly sent to me for inspection by Head-forester TH. PALM, showed yellow under-side even on full-grown specimens. I am sure now that this is a sign of intercross with *elegans*.

In this connection I cannot omit emphasizing that *latescens*, on account of the well-defined outlines of his black colour pattern on a clear straw-coloured ground, would have been more entitled to bear the name of *elegans*, were it not already given to quite another species whose representatives by no means deserve their name as full-grown. As *latescens* is common in Denmark, it may also occur in North Germany, confounded now with *depressus* (♂), now with *elegans* (♀). In that I can see one of the reasons for which the latter species has been placed by several earlier authors as a synonym under *depressus*.

With the conception of *latescens* as a true species the results of the F₁-generation after crossing with *depressus*, detailed above, agree better than they do with the experiences of the usual mendelian race-crossing. As *latescens* is the commonest of these two species in Sweden and in Denmark as well according to J. C. SCHIÖDTE's account in his "Genera og Species af Danmarks Eleutherata" of 1841, it is probably by a mere chance that FABRICIUS laid a pair of Swedish specimens of the not very common *depressus* as basis for the description of the

latter species. His description is so generally written that it agrees very well even with *latescens*. This species is a lake form, while *depressus* is a form preferably found in running water according to the earlier Swedish literature as mentioned above. The slower temperament and the clumsier movements in water of individuals belonging to the latter species are probably connected with their different residence. I myself have not seen *depressus* under natural conditions and I am not inclined to lay stress upon occasional finds in a certain environment. Rivers, streams, rivulets and brooks have sometimes widenings with still water rich in plants which as far as most of the carnivorous Dytiscids are concerned offer much the same ecological conditions as lakes. If, on the other hand, running water in the places for an abundant occurrence of *depressus* is to be understood *verbatim*, the matter stands quite differently. But concerning that, our most closely connected literature is silent.

In 1933 I sent separately to Prof. PLATE in Jena my paper of 1932 and got from him the following declaration. "Ich halte (nach Ihren Angaben) *latescens* und *depressus* für "biol. Arten" (Lehmboden, bzw. fließendes Wasser), die noch nicht "echte Arten" geworden sind, da sie sich noch unter einander kreuzen. Durch die verschiedene Lebensweise haben sie sich verändert, sind aber noch nicht sexuell isoliert." I have quoted this utterance as a proof of what one of the leading geneticists of the present time thinks proper to reply on a endeavour to unveil hitherto overlooked and for the rest inquired results of spontaneous species-crossing in mass within the animal kingdom. In his works PLATE does not deprive the known spontaneously interbreeding species of plants of their authorization as true species. Why this partiality? In the main his quoted utterance reproduces opinions valid a century ago, which already, DARWIN, as mentioned above, rejected. The question was not how the two forms *depressus* and *latescens* had come into the world and what their fate would be. I have plenty of phantasy myself, too. But it interested me to hear PLATE's meaning as regards the following three questions. Is the higher specialization in animals in comparison with plants, emphasized by PLATE and others, the real cause of the prevailing absence of zoological parallels to the many known species-crossings in plants? Are the distinctions made by me according to customary praxis sufficient for separating the two forms in question from each

others as true species? And, if so, ought the intermixture of character specific for each of the two species treated, observed in nature, to be considered as results of an intercross between them, since they agree well with a similar intermixture of characters in interbreedings, duly controlled, of the two species in question? If the answer to these questions cannot be considered as in fact already given above by me, I will abide the time without anxiety.

It would, indeed, be interesting to enter a little more into some details of PLATE's utterance just quoted. As he did not say that *depressus* and *latescens* were differentiated from a third species which neither I, nor anybody else, I think, were capable of pointing out among *Deronectes* now living on the earth, one of the two named must be the phylogenetically older one. From a logical point of view it is only one of these forms whose specific right PLATE denies. *Deronectes* live only in clear and as airrespiring animals moreover only in shallow water because of the neverceasing necessity for them to go up to the surface for a supply of fresh air. For this lakes offer far more chance than running water especially those with a permanent and sufficient amount of water during the whole year, an indispensable condition for the brood. In accordance herewith *latescens* being a lake-form is more common than *depressus* which should be, as mentioned, a form living in running water and being only in small limited places able to give a sure refuge to the brood. The last mentioned species is the rare and more specialized one as regards its ecological conditions, on account of which it is the phylogenetically younger one too. It is in fact against this species that PLATE direct his objections. FABRICIUS described *depressus* in 1775, and the congruence of his species with its representatives of the present time his preserved type-specimens have proved. The form which I called *latescens* was known, as mentioned above, by GYLLENHAAL and described by him in 1808 (Ins. suec.) as a variety of *depressus* but without a special name. Though *latescens* and *depressus* are older than from 1775 when the last species was described, we may start from that year. Till the present time with one generation a year at least, the two species have thus had about 160 generations each, equivalent to the number of human generations from the dawn of the history of man according to conventional estimation. Apart from this parallel, it seems to me that 160 generations together with such a

thorough alteration as the specialization of *depressus* to running water, might suffice to cause at least a slight indication of sexual isolation from *latescens*. But nothing of that is to notice.

In the cited utterance PLATE declared that he considered *depressus* and *latescens* as "biologische Arten", i.e. such ones "welche mehr in der Lebensweise als morphologisch differieren", according to his definition (PLATE 1933, pag. 1065). The differences above detailed as regards certain colour characters together with the divergences described in my quoted papers (1932 and 1933) in all larval, pupal and imaginal stages of the two species in question, amounting doubled together to over a hundred different morphological formations give, I think, full evidence that PLATE's definition of "biological species" cannot be applicable to this case. If PLATE's meaning (PLATE 1933) as regards the phylogenetic genesis of "good" or "true" species is right, the couple of *latescens* and *depressus* seems to have come far beyond the stage of "biological species". But PLATE's (l.c.) next higher stage in the series of evolution is exactly "die guten Arten, die auch äusserlich deutliche Unterschiede aufweisen".

By that the question whether *latescens* is a true species or not, may be considered as duly and affirmatively answered even from a genetic point of view. The result is, that I have been able to prove that true animal species sometimes interbreed in the open and form a more or less dominating population of bastards.

II. *The British Isles*

This is, however, not the only example of species-crossing in the open which I can advance. Already in my paper of 1932, I reported on some other species of *Deronectes* having the same power. Among them I mentioned *elegans* PANZ. not native in Sweden or in the other northern countries. This species occurs according to ZIMMERMANN (l.c. 1933) in Germany, Austria, Switzerland, France and Holland. According to BALFOUR-BROWNE it occurs also in the British Isles though confounded with *depressus* and is in fact the commonest of the two, because of which it is often called by him the "common species". BALF.-B. gave in two papers (1919 and 1930, see References) accounts of his investigations which are based upon a very

extensive material, finally about 700 specimens, captured in many different places in England, Scotland and Ireland. He had found that in northern England, in Scotland and Ireland *depressus*, but in south England *elegans* prevails. In an intermediate zone he found specimens agreeing as to their aedeagus neither with *depressus* nor with *elegans*, exhibiting mostly an intermediate form. The former one, I may add, has an aedeagus with broad, spatulate end, the latter an awl-shaped one. In the beginning of his researches there was for a long time a gap in the middle of the series shown by the examined aedeagi. Finally he found in Loch Dungeon specimens filling up the gap so that he got an unbroken series of aedeagi from awl-shaped to spatulate. In the paper of 1919 (l.c.) in which he had the closed series reproduced he writes: "The first explanation which will occur to any one is that we have in Loch Dungeon a hybrid; but there are one or two objections to this view. In the first place, I did not find in the loch any male with an aedeagus of the normal common species' type." — — —. "In the second place, if this loch contains hybrids, why does no other of the thirty-two lochs I have examined contain them?" — — —. "It seems open to question, therefore, whether we have merely one species showing extreme range of form or whether we have two species very closely related to one another. On the evidence in my possession, i.e. after examining considerably more than five hundred specimens, I am inclined to adopt the latter view." — — We see, thus, that BALF.-B. had meditated upon hybridization but rejected this idea. In his later paper (1930) founded on new investigations of further material, he only in passing took up the question of hybridization, not suggesting any change of his opinion. On the other hand he no longer accepted *elegans* as a true species but put it simply as an aberration under *depressus*.

Having been informed of BALF.-B.'s publications mentioned above I saw at once that he had not been able to understand what an excellent find he had made. On the contrary, he had rejected the only correct interpretation of his find for reasons not convincing for a collector of Dytiscids accustomed to work in the field. As I pointed out already in 1922 in my paper (Stockholmstraktens Vattencoleoptera, Entomologisk Tidskrift 1922), one cannot know beforehand the contents of Dytiscids in the different waters even if these appear to be rather uniform. If one makes running researches in the same lake

and even at the same place on the shore, for instance every month, from the breaking up of the ice in spring to the covering with ice in autumn, as I have made, one will find that Dytiscid species come and go. Likewise one will find that the different places on the shore of the same lake show different species according to the prevailing direction of wind, sunshine and shade, to the vegetation, to the bottom and so on. Because of that I contend that *elegans* may very well have been present in a lake on a certain occasion without coming into BALF.-B.'s net. Further I may ask, if a certain lake had really contained bastards, why should the same water at the same time contain the "common species" (*elegans*), i.e. the one of the two interbreeding species in pure state? It has once been there in such a state and left marks of itself in the bastards. Besides, the appearance of a population of bastards, at least for a considerable time, depends upon what species is the native one locally. The invading species is as a rule in the minority.

As to the second objection to the idea of hybridization it proves that BALF.-B. did not know a real *depressus* which never shows the rounded form of aedeagus-end reproduced in BALF.-B.'s figures. Moreover, these are not true to nature, disregarding some important details in the anatomical structure of the aedeagus. In *depressus* the apex of aedeagus is constantly broad, at the very tip square, towards the middle of the whole organ slightly narrowing, provided with a broad, striated, stronger chitinized margin, even at the tip. In *elegans* the apex is constantly awl-shaped, at the tip blunt, towards the middle gradually widening with a straight, narrow, strongly chitinized margin. The differences of aedeagus in these two species are of such a striking nature that BALF.-B.'s proposal of placing *elegans* under *depressus* as a mere aberration is a clear blunder, were he not to be excused, to some extent at least, on account of his mixed material. I refer for the rest to the drawings in my paper (FALKENSTRÖM 1932) as regards the aedeagus of *depressus* and *elegans* and to Plate II, figg. 3-5 of this report.

In his paper of 1919 BALF.-B. gives figures, as mentioned above, of the closed series of the aedeagus of his *depressus* and *elegans* which I have allowed myself to reproduce here (see textfigure 1). I suppose that the ends of the series, i.e. fig. 1 and fig. 18, reproduce the form of aedeagus in respectively *depressus* and *elegans* according to BALF.-B.'s

meaning. While fig. 18 gives the outlines of the organ in *elegans*, fig. 1 does not at all reproduce that of *depressus*. The original is a clear bastard. Likewise the organs, fig. 7 and 8 on his plate VIII and all claws on it belong to bastards. Moreover, the figures in toto on plate VII show the more or less hybrid nature of the originals as to different details, the fig. 4 shows nearly *latescens*. As BALF.-B. presumably

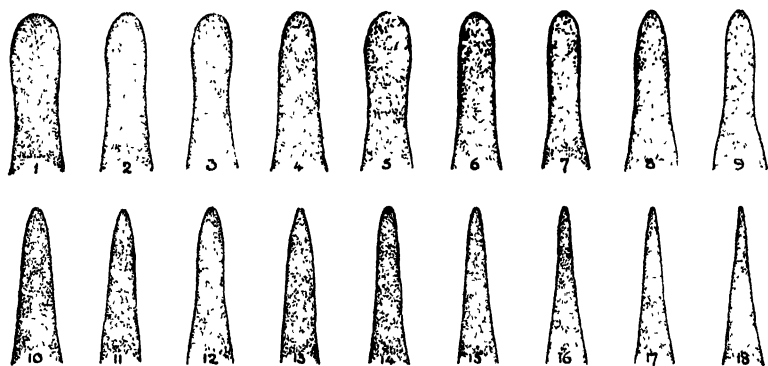


FIG 1 Reprint from Balfour-Browne's cited paper of 1919, in Ann and Mag Nat Hist, ser 9, vol III, p 297

Apices of aedeagi of specimens of *D. depressus* („the northern species”) and *D. elegans* („the common species”), chosen to show the range of variation

Figs 1- 9 *D. depressus* (1, Talkin Tarn, 2, L. of the Lowes, 3 L. Uri, 4, L. Doon, 5 9, I. Dungeon) Figs 10-18 *D. elegans* (10, 11, R. Spey, 12, 13, Long L. of the Dungeon; 14, L. Stroan, 15, L. Aber, 16, L. Skene, 17, Broadford River, Skye, 18, Moorlinch, N. Somerset)

selected the originals of his figures, except the transition forms of the aedeagus, in such a manner that he took the most extreme ones, i.e. the types of *depressus* and *elegans* according to his understanding, it seems to be hopeless to search longer for pure *depressus* in the British Isles.

The main points of my critique of BALF.-B.'s papers I emphasized in my paper of 1932 together with my astonishment that BALF.-B. did not check his material by comparison with specimens from abroad instead of conferring to *depressus* an amplitude of variation which *a priori* may be considered to be suspiciously large and depriving *elegans* of its rights as a true species. Our Dytiscid Systematics is certainly not so lax that alterations of so radical a nature can occur unchallenged. In my paper (FALKENSTRÖM 1932) I therefore did not

accept BALF.-B.'s measures, being quite sure that every systematist of this branch now and in the future will share my point of view.

The accounts in my quoted papers of 1932 and 1933 of BALF.-B.'s investigations in question and of his conclusions which I, as mentioned, criticized in a thoroughly benevolent way, seem to have roused his feeling, since he in a later paper (BALFOUR-BROWNE 1934) subjects my cited papers to a very malicious critique in which he advances a wrong citation (i.e. p. 45), containing the contrary to my text, employs my words as arguments against me in quite another connection, distorts and derides my statements and so on. He reaches the culmen in his paper by rejection of the results of my investigations though the neither had seen *laetescens*, nor knew anything about its metamorphosis or of that of *depressus*. It seems to me that BALF.-B. instead of getting angry ought to be grateful to me for having placed his investigations in a light which they never could have attained by themselves.

In his last-named paper BALF.-B. still treats an impure material, evident by his figures, and attempts to depreciate the genital organs as criterion of true species, quoting some authors, among others also a couple in *Lepidoptera*. Already DARWIN (Or. of Sp.) has pointed out that characters useful for systematic purpose are different within the different groups of animals. A certain part of the body is in some animal groups constant and, therefore, useful as characteristic, in other groups the same part is variable and useless for the purpose. D. SHARP, M. REGIMBART, A. ZIMMERMANN, R. SCHOLZ, L. GSCHWENDTNER, myself and many with us have found that the male genitalia in Dytiscids are constant and excellently fit for separating species and even higher systematical units. In *Halipilidae*, *Gyrinidae*, *Cryptophagidae*, *Ipidae* and many other families of beetles reliable Systematics concerning the majority of species is not possible without using the genitalia. The number of beetle families continually increases in which this hardly accessible and previously neglected criterion must be resorted to in order to settle the systematical difficulties. It is thus the experiences which decide the availableness of the genitalia as differentiating moments.

In one of BALF.-B.'s quotations concerning *Lepidoptera* the author says: "It is idle to maintain that geographical representatives are specifically distinct, if their sexual armatures show obvious differen-

ces." Knowing too little about the systematical points within this group of insects I am not able to judge the value of this statement. But, as shown, it has no universality. In Dytiscids I have found that old recognized species with very extended distribution can show a different form of the male genitalia within the different areas of fauna together with minute superficial differences, previously not observed or accentuated. But I have also found that old species likewise with extended geographical distribution show completely uniform characters, superficial and sexual within the whole occurrence. *By that it is evident that geographical distribution in itself is not alone decisive.* A distinction must, however, be made from a systematical point of view, and in the first case I have divided the old species. Further we have the many species of more or less extended geographical distribution which in their superficial characters are not distinctly separable but show clear differences in their male genitalia and, lastly, there are the forms with equal genitalia but different superficial qualities, often as regards colour, colour pattern, size, hairiness and so on. If in all these cases the sexual character proves constant, we let it decide whether a species or only a variety, an aberration, or an acceptable extent of variability within the species in question is before us.

In order to get, if possible, full evidence in an experimental way as in the case of *depressus* and *lutescens*, that the great variation observed by BALF.-B. in the form of aedeagus in his *depressus*-complex depends upon interbreeding of *depressus* and *elegans*, probably also of *lutescens*, I ask my friend and colleague Dr GUIGNOT, Avignon for living material of *elegans*. With his usual ready courtesy he sent me a collection from the vicinity of Nancy in 1934. A couple of females immediately laid eggs from which I raised two series of larvae developed as usual under individual control into imagines. The following year I crossed males of *elegans* of these series with females of my stocks of *depressus* and *lutescens*, three crossings of each kind. From these 6 pairs I got, with individually different facility within the respective pairs, series of bastards some of which I let accomplish their metamorphosis to full-grown beetles. In the main it proved to be a little more difficult to get result in the crossing of *elegans* and *lutescens* than in that of *elegans* and *depressus*. Having

later on in the autumn of the same year obtained fresh material of *latescens*, I put together a male of *latescens* and a female of *elegans*. From this crossing I got offspring in the present year. As I want to see how the F_2 -generation will appear, I must spare my material on account of which I am forced to put off a detailed report of the obtained bastards to a later occasion. I can, however, shortly report that by breedings in the present year of the F_1 specimens (bastards from 1935 and 1936), partly by pairing *inter se*, partly by back-crossing with one of the parents, plenty of progeny were produced. I restrict thus my account now to a reproduction of some aedeagi of the F_1 bastards. (See Plate I, figgs. 7-8 and Plate III, figs. 1-4). Already from these figures it is evident that the form of aedeagus varies individually, that it is not equal either with the typical form of *elegans* or that of *depressus*, resp. of *latescens* but that it exhibits intermediate stages between the different forms of the parental organs, obvious correspondences to some of BALF.-B.'s figures in his closed series of transitions. By that it seems to me as proved to a certain degree that my conception of the hybrid nature of the variable form of aedeagus in BALF.-B.'s material, expressed in my paper (l.c.) of 1932 was quite correct. On account of this it was, of course, also quite out of question to lay it as a basis for alterations of *depressus* and *elegans* in systematical respect. As BALF.-B.'s explorations in the open are so extensive it will appear as if the spontaneous interbreeding of *depressus* and *elegans* had already considerably altered the fauna of the British Isles as to these two species. Nothing gives one the right to consider this fact as unique in animals. Instead of the unimportant rôle which interbreeding of animal species in nature are now considered to play, it is very possible that in the future, according to further explored instances, the views will be essentially altered. As set forth in my

paper of 1932 (l.c.) and later on in my paper of 1935 (l.c.) nearer manifested, the spontaneous species-interbreeding in animals have certain qualifications for rendering service in the raising of new forms. These could then be fixed as to cause and progress without the help of other hypotheses than that of the presence of genes. This would be a great benefit, moreover gained within a range outside that of Genetics up to now, at least as regards Zoology.

III. *Other parts of Europe*

These species-crossings proved by me for Sweden and for the British Isles, which have been so thoroughly misunderstood by BALFOUR-BROWNE in regard to the latter, seem to have full agreement within the rest of Europe according to a paper just sent me by the author, Dr P. FRANCK, Hamburg (1935), and entitled: "*Deronectes depressus* F. and *elegans* PANZ. als Rassen einer Art". On the plea of BALF.-B.'s above mentioned papers (1919 and 1930) FRANCK advances the results of his own investigations of more than 500 specimens of which about 230 are males of his own and others collections. These contain specimens from Germany, Austria, Hungary, Holland, France, Spain, Denmark, Norway, Sweden, Finland and Russia. The main content of his explorations, illustrated with three series of reproduced outlines of ends of aedeagus from 33 specimens (See textfigure 2 hereby), he sums up thus: "Wir haben also das typische Bild einer Kaferart, die zwei geographische Rassen herausgebildet hat. Um dies scharf zum Ausdruck zu bringen, muss das ganze Gemisch einen gemeinsamen Namen erhalten, wofür der älteste Name *Deronectes depressus* F. in Frage kommt. Die beiden Rassen sind dann als subspecies *depressus* F. und subspecies *elegans* PANZ. zu bezeichnen. *Deronectes depressus depressus* ist die Nordost-rasse" — — — "*Deronectes depressus elegans* ist die Südwest-rasse" — — —. "Für die Zwischenformen bringe ich die Bezeichnung *D. depressus intermedius* in Vorschlag." — — —

The old, long ago recognized species *depressus* and *elegans* the author will thus replace by two races, each ternarily named, and all intermediate forms he groups together under another ternary name. As basis for his threefold division he lays the variable form of the ends of aedeagi under fair admission of the fact "dass bei dieser Abgrenzung eine gewisse Willkür unvermeidlich ist".

If anything in Systematics must be avoid, it is above all arbitrariness. The old sentence "in dubio non est agendum" is in the highest degree applicable within this discipline since doubtfulness bears arbitrariness and this spoils Systematics which has to deal only with objective realities. If between two or more groups of organisms a thorough distinction is not possible in an objective way, the groups in question belong genetically to each other and must not be separated. Incomplete distinction within a species, i.e. different formation of a certain more or less variable character constitutes aberrations and varieties. Old recognized species with extended geographical range could, as mentioned above, be suitably separated in more species on account of the different formation of the aedeagus. But in these cases there were no connecting transitions between the different formations. From the beginning of Systematics connecting transitions have made obstacles for the raising of new species from the forms in question. In mammals and birds races under ternary or more denominations have in later time been raised as lowest units in order not to alter too much the old Linnéan names. Such races are from a systematic point of view largely equal to species in other groups of animals and might be binarily denominated as well. The Systematics founded by LINNÉ has not profited by those short-sighted measures. On the contrary, they would break down or at least shake his aimed systematic building, if they were followed also in other branches of Systematics. Moreover, races as appertaining to human domestication of plants and animals including the explorations of Genetics, have nothing to do with wild organisms. (See further thereover in my quoted paper of 1935).

Some biologists like to put forth divergent forms as geographic races. In these cases the original species is known. A thorough examination will usually reveal the divergences sufficient to constitute different species as already mentioned. In other cases the divergent forms are variations or aberrations in a single character from the known species, as mentioned above as well. Geographic races do not serve any systematic purpose. FRANCK's declaration that a diagonal line through

Germany separates his north-eastern race from his south-western one is of course not sufficient to compensate for the lack of distinct morphologic divergences between the two.

Apart from these principal objections to the form of Systematics

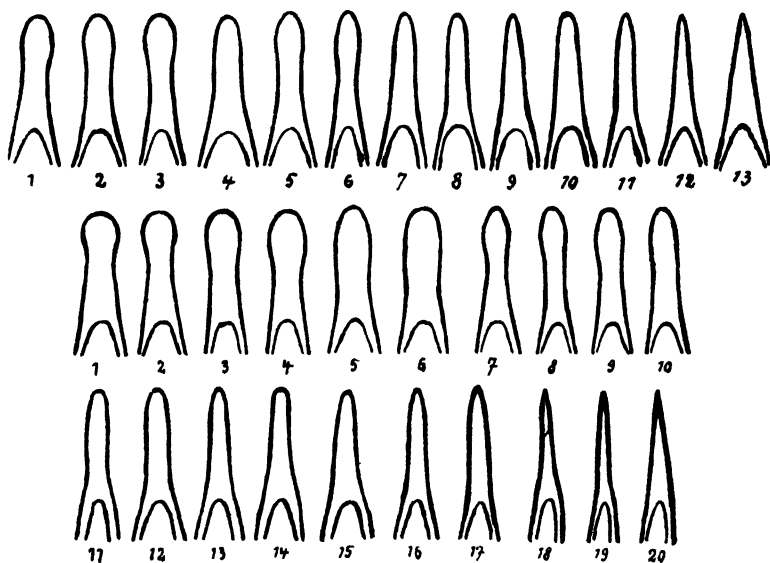


FIG. 2. Reprint vom FRANCK's cited paper of 1935, in Entomol.-Blätter Jahrg. 31, 1935, p. 238

Erste Reihe Material von Bad Bramstedt in Holstein Zweite und dritte Reihe Exemplare aus verschiedenen Gegenden Europas

advanced by FRANCK I will add some of more practical nature. According to the heading of FRANCK's paper, *depressus* and *elegans* should be races of a species. Unfortunately he did not tell us from which species they had arisen. They must, of course, have an origin. That a species under new and altered conditions can make new forms arise, we have learned already from DARWIN. But it is a mere supposition. Likewise, the author leaves us in the dark about the altered conditions producing his two races in question. Under domestication it is known that crossing can produce new forms. At all events, a mere declaration of *depressus* and *elegans* as races of a species is of very little value for Systematics.

The unspared labour which FRANCK apparently has bestowed upon

his investigations is worth every recognition. Perhaps a more careful study of the literature would have been of value for him. In the literature which he quotes he has not mentioned my two papers (l.c. 1932 and 1933), though these are recorded in Bibliographia Zool. and in Zool. Record. Otherwise he would have found out himself that it is the species *latescens* which I have separated, which has caused the greatest difficulties for himself. ZIMMERMANN and others to get a good grasp of *depressus* and *elegans*. As my *latescens* with regard to some characters resembles *elegans*, with regard to others *depressus*, it is evident that it has fair qualifications to mislead. FRANCK's reproductions of the end-parts of aedeagus show that he had not the real *depressus* as originals according to reasons mentioned above in my critique against BALFOUR-BROWNE. Likewise, FRANCK's description of the male fore-claws in *depressus* is wrong, since both fore-claws in this species are elongated, scythe-shaped, at the end abruptly bent. His description agrees completely with the fore-claws of *latescens*. As the form of aedeagus and that of the fore-claws are the two most important promises to his conclusions, it is not surprising that they also are wrong. Summing up the main points of FRANCK's attempt to solve the *depressus* problem raised by BALFOUR-BROWNE, I dare say that this has not succeeded. It is really astonishing that FRANCK did not take up hybridization as a cause of the transition forms, but follows lines which must necessarily lead astray.

To get a true result of these investigations, the distribution of *latescens* in Europe must be explored. In Sweden, Denmark and Finland it is the most common species and *depressus* the rare one of the two, as already mentioned above. There is all probability that *latescens* is represented in a great part of FRANCK's material and that all three species have participated in the appearance of some of the specimens examined by him.

I reject, thus, FRANCK's peculiar idea of *depressus* and *elegans* as races of a species, by him not detailed and not even known. Now as before the two are true species, excellently well separated from each other by their normally constant, very different aedeagus. They possess, however, power of interbreeding. The transition forms, some of which FRANCK called *D. depressus intermedius*, are bastards and as such are not qualified for the system of this insect group according to actual rules, on account of which priority cannot be claimed. They

must instead be indicated as usually, for instance *depressus* \times *elegans*, *depressus* \times *lutescens* or vice versa.

Referring to another paper with a detailed report of the metamorphosis of *elegans* and of the obtained results of crossing between this species and *depressus*, respectively *lutescens*, I will conclude my present paper by emphasizing the interesting fact that *Deronectes depressus* F. (1775), *elegans* PANZ. (1794) and *lutescens* mihi (1932) have proved to interbreed in nature, giving the result that the bastards within great parts of Europe have become predominant to such a degree that the pure species have been displaced. Species-crossing is thus a factor to include in judging a fauna, especially concerning species with more pronounced variability.

Finally I must express my sincere gratitude to my collaborators, Dr. N. ODHNER of our State-Museum (Riksmuseum) here and Mrs. EVELYN HÖRVER at Uppsala for their unspared labour, the former with the excellent original photos, the latter with the retouch of my English.

SUMMARY

1. Interbreedings of animal species in nature resulting in fertile offspring in mass is so far unknown. Above I have shown that three well-defined species of *Deronectes* (waterbeetles) intercross two and two in the open resulting in the displacement of the pure species and in the predominance of the bastards in many parts of Europe. In another place quoted above I have reported on the intercrossing of two other species of *Deronectes* in the northern parts of Norway and Finland. Here the common bastards were mixed with luxuriant ones, so-called gigas specimens, probably results of polyploidity, together with specimens like the true species. In another paper also quoted above I have reported on a mixed population of bastards of two species of *Hyphydrus* (waterbeetles) native in China, together with a few specimens of the true species in question. Above I was also able to report shortly on a fully spontaneous intercross of two highly different species of *Drosophila*-flies giving a F_1 -generation whose mixed nature corresponds very well with the results of my beetle

crossings. As the mating flies, *Dr. fasciata* (= *melanogaster*) ♂ × *Dr. funebris* ♀ are common all over the world, it is just possible that intercrossing between them may occur anywhere. Under such circumstances and because of the fact that the male bastards of the spontaneous cross in question were extremely like the male of the pure species of *fasciata* one is prone to doubt whether the stocks of "melanogaster" in culture in the laboratories are always homozygous. The labile state of "melanogaster" in cultures can be caused in this way, a parallel to *Oenothera* in any ways.

2. The main result of such species crossings seems to be a very striking increase of the individual variation. Characters constant in the pure species and because of that used in systematics as distinguishing moments, exhibit in the bastards a high variability due to which incorrect determinations have repeatedly been made even in the present time as shown above concerning England and Germany.

3. The fact long ago known that plants and animals consist of fairly constant species mixed with plain variable ones will surely have one of its most active causes in the different power of crossing in the open.

4. As animal species crossings in the open controlled and studied in crossings of the same species in culture deserve no doubt more common interest on the part of biologists, I thought it necessary to give full particulars of my work and of its results. For that reason I have described and tabulated above some reliable characters in the pure species as well as in the bastards. These characters refer thus not only to the ready beetles but also to the 3d stage of their ontogenetic development, in many cases even to that of one and the same animal — a fact without parallels I think in zoological literature. The pure species of my stocks were subjected to examination of their homozygosity during many years by means of strong interbreeding sometimes completed by back-crossing with the parental generation.

5. The species-crossings studied do not show congruence with the common race-crossings as to their respective results, which I think can never be expected, as two different species and two races of a species are, in fact, incommensurable. For the same reason it is senseless to compare crossing results of more or less damaged individuals from the laboratories or of cultivated animal races with those of natural species. Likewise it is inappropriate to generalize

results attained in plants to animals without performed investigations of the animal in question.

6. It is shown above that colour and colour pattern in beetles are hereditary, i.e. founded in the bodily constitution and not due to environmental conditions as most genetic authors affirm.

7. Although we have learned in Genetics that homozygous races breed constant under unaltered conditions, many geneticists are inclined to deny the existence of constant species in nature. Every systematist knows by experience that plenty of such are to be found there.

8. The living things show in their bodily construction traces of congruence and of incongruence. They have equal or different characters due to which they can be arranged in groups, so-called systematic units of lower or higher degree, which stand in animals, respectively in plants in subordinate position to each others. These groups or units are based upon visible and measurable, thus real things, viz. the characters of the individuals which belong to the units in question and are because of that realities themselves, which fact is denied by many scientists probably on account of insufficient experience in systematic work. LINNÉ understood the importance of this fact and arranged his systems accordingly and so have all his followers.

9. The lowest unit is the species which consists of all similar individuals, completely limited from the individuals of all other species by means of their characters. The higher units (genus, tribus, family, class, ordo, phylum and so on) are built up likewise only by means of the characters of the individuals which belong to the respective units and they are, thus, even real things. According to the ground of classification the units can be altered in regard to their contents and new units arise with equal reality as have the former.

10. The fact that one can read off the whole series of units from the base to the top to which an individual belongs only by examining his bodily characters, proves the rationality of the scientific system. But not only that. It shows also the genealogical descent of the individual in question. The living things bear on and into their own bodies the undeniable proofs of the truth of the doctrine of evolution. Congruence of characters in the living things can only have been realized in an unbroken series of offspring from the origin of the form

in question down to the present time. I emphasize also that reality of systematic units and constancy of species are indispensable premises for the doctrine of evolution, the cardinal point of biological science.

11. Although LAMARCK's and DARWIN's doctrine of evolution has stood the test, their hypotheses for its grounding on the other hand have not held, as for instance the heredity of acquired characters and DARWIN's postulate that all species were highly variable in their essence. As to the latter I have shown above that it has no support from a systematical point of view. DARWIN's doctrine of natural selection rests upon a minority of animal species whose members show sufficient power of variation. Only by heterogeneity and irregularity of the cell-dynamic or as a reaction against altered environmental conditions does the variability arise.

12. I have shown above that constancy of the germ plasm, i.e. the genes, has as a consequence constancy of the species, respectively of its races. Irregularities in the chromosomal dynamic, crosses of heterogenic individuals, strong fluctuations of the environmental conditions and so on, are sufficient to alter the species and their races in a natural way.

13. I find it necessary to distinguish between the environment of the individual and that of the genes, i.e. the contents of the cell. Only the latter has importance in the development of the cell.

14. The environmental conditions act as stimulants and have no specific effect on the being of an individual. On account of that it is absurd to speak of the effect of environment separate from the reaction of the genetic constitution. The latter is of course alone responsible for the effect within the organism, even if the former stimulates it. The general physiologic responses of the animals to environmental conditions are not commensurable with the responses caused by environment during the whole genesis of the individual and its cells. Because of that I emphasized above that the genetic doctrines of the relation between environment and the living things may be subjected to a radical review.

15. According to the gene-theory the genes must be living particles, thus, capable of nutrition, growing, division, etc. By that the way is open for the environmental conditions of different kinds to co-operate in building up the internal construction of the genes, eventually with the result that the genes or some of them be altered in accordance

with the residence of the individuals. In this way the undeniable adaption of the individuals to their residence could have its reasonable explanation. Such adaptations need not always to be hereditary, they remain as long as the residence rests unaltered.

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¹⁾ I take the opportunity to make a correction on page 180 of this paper where, in the middle, the words „gar nicht“ must be changed to „ja, ganz gewiss“.

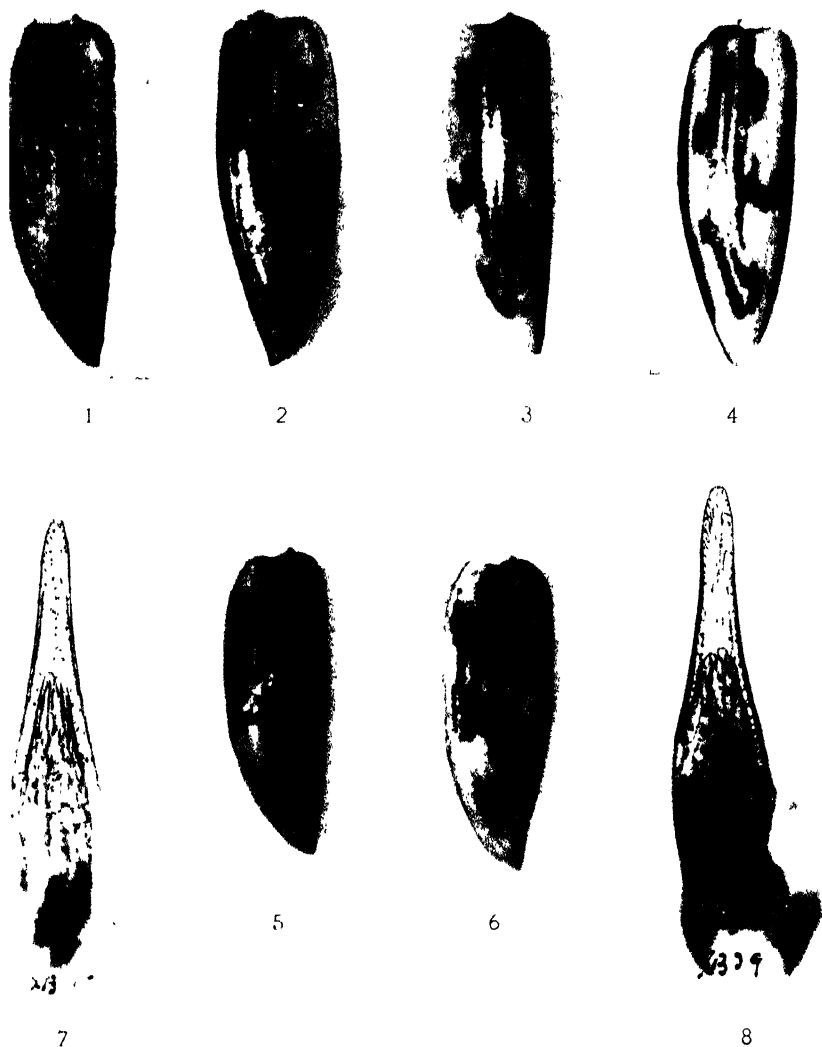
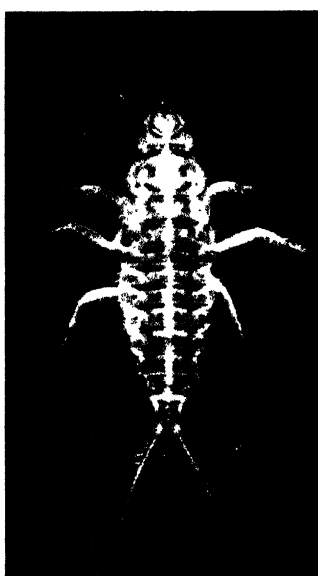
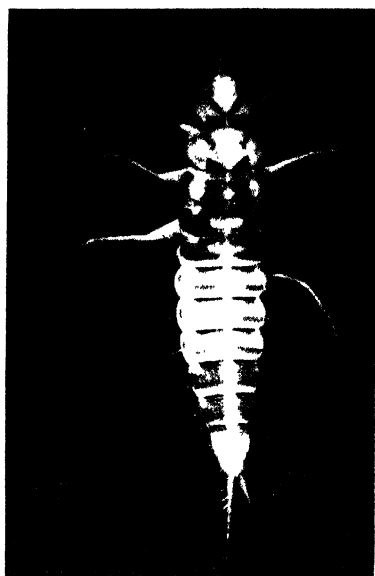


Plate III. FIG. 1 and 2 show the wing-cases of *D. depressus* F₁, magnified 11,5 \times , fig. 1 from above, fig. 2 from the margin.

FIG. 3 and 4 show the wing-cases of *D. latescens* FARK, magnified as before, fig. 3 from above, fig. 4 from the margin.

FIG. 5 and 6 show the one wing-case of *D. elegans* PANZ, magnified as before, fig. 5 from above, fig. 6 from the margin.

FIG. 7 and 8 show aedeagus of bastards (brothers), F₁-generation of 1935 by crossing of *elegans* δ \times *latescens* δ . Magnified 90 \times .



3

4

5

Plate IV. FIG. 1 Larva (depr. II 4 1932), 3d instar of *D. depressus*, pure species of my stock, magnif. $9\times$ — FIG. 2 Larva, 3d instar of *D. latescens*, captured in the open in 1935, magnif. $9\times$. — FIG. 3. Aedeagus of *depressus*, pure species of my stock in 1930, (depr. 21, 1930), magnif. $90\times$ — FIG. 4. Aedeagus of *elegans* from Nancy (France) in 1934, pure species, magnif. $90\times$. — FIG. 5 Aedeagus of *latescens* from "Saby-viken" in 1935, pure species, magnif. $90\times$.

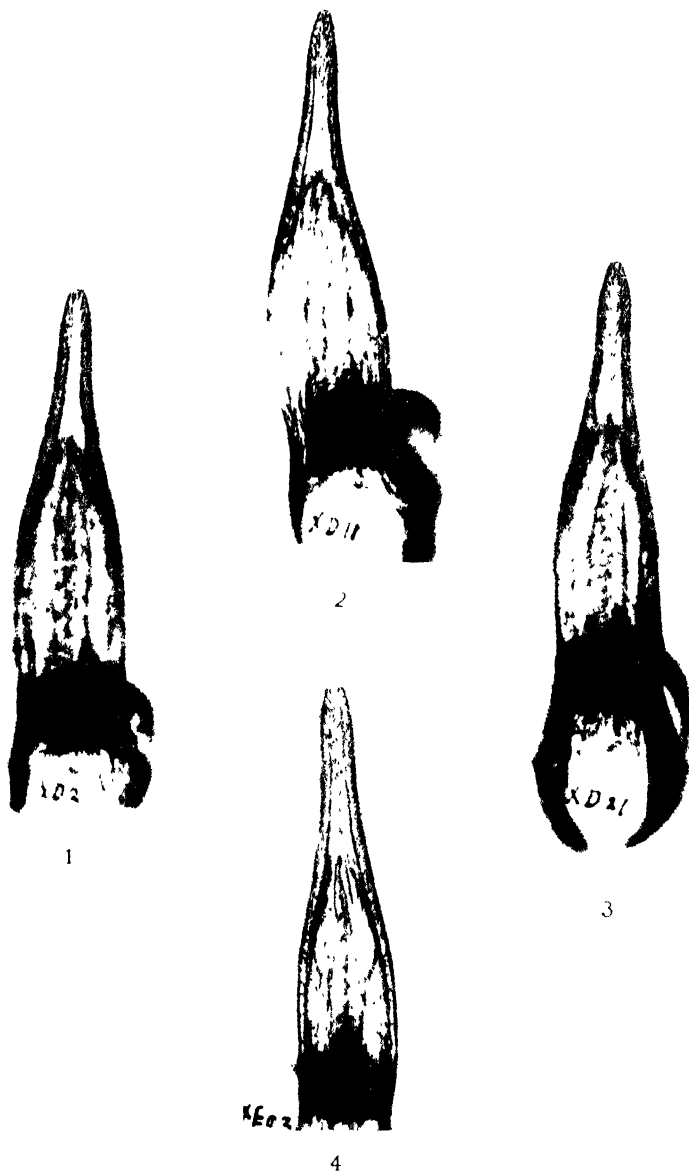


Plate V. FIG. 1, 2 and 3 show aedeagus of bastards (brothers), F₁-generation after crossing of *elegans* ♂ and *depressus* ♀, magnif. 90 ×. FIG. 4 shows aedeagus of another bastard series, F₁-generation after crossing likewise of *elegans* ♂ and *depressus* ♀, magnif. 90 ×.

UNTERSUCHUNGEN ÜBER MUTATION BEI TABAK

II. EINIGE KÜNSTLICH ERZEUGTE CHROMOSOM-MUTANTEN

von

D. TOLLENAAR

Soerabaja, Java

(Manuskript eingegangen am 10. August 1937)

Mit 4 Tafeln

Bald nach Abschluss des ersten Teils dieser Veröffentlichung über künstliche Mutation war die Krise Ursache dafür, dass ich meine Arbeit an der Tabakversuchsstation Klaten und damit auch diese Untersuchungen abbrechen musste. Auch anderswo konnte ich sie nicht beenden, während andere sie ebensowenig hätten weiterführen können.

Ich habe mich daher jetzt entschlossen, wenigstens einige der erhaltenen Ergebnisse kurz abzufassen.

Alle Chromosom-Mutanten wurden durch 10 Minuten-Bestrahlung auf 35 cm Abstand mit 50 KV und 3 mA von Blüten einer völlig homozygoten konstanten Tabaklinie erhalten. Für weitere Einzelheiten verweise ich nach dem ersten Teil dieser Veröffentlichung (3).

Die Beschreibung der Chromosomen-Situationen ist auf Studien an Pollen-Mutter-Zellen (PMC) nach Fixierung und Hämatoxylin-Färbung begründet.

§ 1. *Übergang zwischen Gen- und Chromosom-Mutation*

Schon in der ersten Abhandlung wurde darauf hingewiesen, dass eine Scheidung zwischen Gen- und Chromosom-Mutation oft recht künstlich ist. Ein Beispiel einer Mutation, welche einen derartigen Übergang zwischen Chromosom- und Gen-Mutanten bildet, zeigt uns *oligofolia*.

Oligofolia

Eine wenigblattrige Tabakpflanze (± 20 Blätter), daher frühblühend; Blätter bläulichgrün.

Nr	Selbstung	Ausserliche Beschaffenheit der Nachkommen	
		<i>typica</i>	<i>oligofolia</i>
3832	F_2 F_1 -Pflanze	30	8
104	F_3 <i>oligofolia</i>	0	114
105	<i>typica</i> aus F_2	57	0
106	dasselbe	41	16

Also wegen der Mendelspaltung eine deutliche monofaktorielle rezessive Mutation

Die Zahl der Chromosomen war unverändert 24_{II} (Zählung in der ersten Metaphase und Telophase) Während der ersten Anaphase

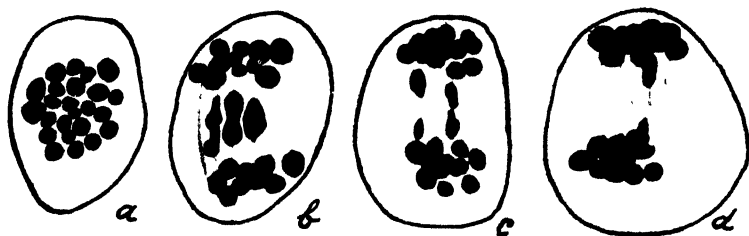


Fig 1 *Oligofolia*, P.M.C

a. Erste Metaphase 24_{II} Chromosome, b Drei Paare Chromosome nachhinkend, c Noch zwei Paare nachhinkend, d Noch ein Paar nicht ganz am Pol angelangt (das Paar mit zugespitzten Enden), späte erste Metaphasen

kann man aber bei *oligofolia* regelmässig Nachhinken von Chromosomenpaaren, sogenannte Retardierung, beobachten und zwar in der Weise, dass 3 Paare Chromosome schwieriger auseinander gehen, wobei speziell ein Paar Chromosome mit zugespitzter Form am längsten

zurückbleibt (Fig. 1a, b, c und d). In der ersten Telophase war die Chromosomzahl schliesslich überall normal 24. Weil aber sichtbare Veränderungen im Verhalten der Chromosomen auftreten, könnte man hier ebensogut von einer Chromosom-Mutation reden.

§ 2. Die *lancifolia*-Mutation (Typus: $2n-1$)

Ein schönes und lehrreiches Beispiel zeigte die *lancifolia*-Mutation. In der F_1 (der bestrahlten *typica*) wurde diese Pflanze aufgefunden, welche von der *typica* durch etwas schmalere Blätter mit mehr aufrechtem Stand und durch grössere Blattzahl abwich (im Mittel 39 statt 32 Blättern bei *typica*). Auch war die Blattfarbe dunkler grün und die Blütenfarbe dunkler rot; die Früchte hatten eine mehr zugespitzte Form. Der ganze Habitus erschien spitziger und die Staubfäden waren etwas kürzer als der Griffel (Fig. 4) Der Pollen war für einen grossen Teil auffallend klein (Fig. 2a) und dessen Inhalt zusammengeschrumpft, er keimte nicht in Zuckergelatinelösung. Es wurden folgende Spaltungszahlen erhalten:

Nr	Selbstung u Bastardierung	Beschaffenheit der Nachkommen		
		<i>typica</i>	<i>lancifolia</i>	sekundäre Mutanten
<i>F</i> ₂				
776	<i>F</i> ₁ -Pflanze geselbstet	485	615	7
776a	<i>F</i> ₁ -Pflanze × <i>typica</i>	466	491	10
776b	<i>typica</i> × <i>F</i> ₁ -Pflanze	887	9	4
<i>F</i> ₃				
15 Pflanzen	<i>typica</i> aus <i>F</i> ₂ geselbstet	2140	0	7
15 Pflanzen	<i>lancifolia</i> aus <i>F</i> ₂ × <i>typica</i>	1026	1059	3

Die Spaltungsverhältnisse stimmen völlig überein mit den beschriebenen Fällen von ewigspaltenden, nur Gen-mutierten Mutanten (z.B. *atroviridis*, Seite 130, Mutation bei Tabak I).

Die homozygote Form entsteht also wieder nicht, wahrscheinlich deswegen, weil diese Form nicht lebensfähig ist. Es entstehen nämlich bei der Kreuzung *typica* ♂ × *lancifolia* ♂ wohl 1% *lancifolia*. Die Ursache dieses kleinen Prozentsatzes wird wohl die sein, dass der grösste Teil dieses Pollens nicht keimfähig ist (die kleinen Pollenkörner). Die mikroskopische Untersuchung der *lancifolia*-Pollen-Mutter-Zellen zeigte, dass die Chromosomenzahl nicht 24_{II} , sondern $23_{II} + 1_I$ ist, was in zahlreichen Fällen immer wieder festgestellt werden konnte. Am deutlichsten ist dies während der ersten Metaphase und der frühen ersten Anaphase wahrzunehmen, wobei das univalente Chromosom sich schon voraus nach einem Pole bewegt (Fig. 2c und f).

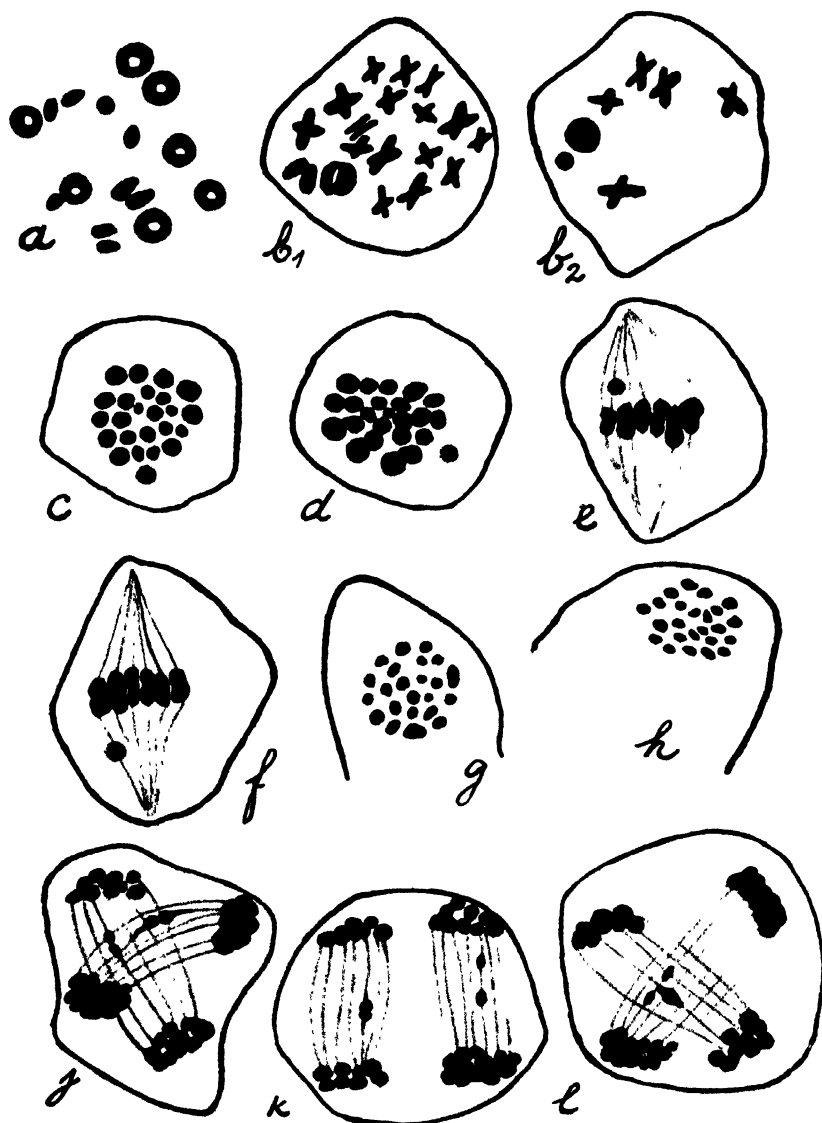
Dieses univalente Chromosom wird dabei in den peripheren Bahnen angetroffen. Es ist, auch wenn alle Chromosome sich während der Metaphase in der Äquator-Platte befinden, an der weniger intensiven Färbung mit Hämatoxylin (Entfärbung mit Eisen-Alaun) den anderen Chromosomen gegenüber leicht zu erkennen (Fig. 2c und d). Auch während der Diakinese sind die 23 Bivalente deutlich von dem einen Univalent zu unterscheiden (Fig. 2b₁ und b₂). In der ersten Telophase wurden am einen Pole 23, am anderen Pole 24 Chromosome wahrgenommen (Fig. 2g und h).

Soweit haben wir eine sehr grosse Übereinstimmung genetisch und cytologisch mit der von CLAUSEN u. GOODSPEED (2) beschriebenen spontanen $23_{II} + 1_I$ -Mutante „fluted“, wobei auch die „fluted“-Eigenschaft nur in 2% mit dem Pollen übererbtete.

Bei der Röntgenbestrahlung ist in einer Geschlechtszelle also eines der 24 Chromosome zugrunde gegangen, wodurch die $23_{II} + 1_I$ -Mutation künstlich hervorgerufen wurde. Es muss aber noch eine weitere Veränderung hervorgerufen worden sein, obwohl dies nicht zu Veränderungen im Habitus der Pflanze geführt hat.

Bei der frühen zweiten Telophase ist nämlich in einem Teil der *lancifolia* stets ein Hinterbleiben von je einem Paar Chromosome zu beobachten (Retardierung), obwohl, soweit das Material studiert wurde, noch immer eine Trennung zustande kam.

Weil diese „Retardierung“ bei beiden Zellteilungen der zweiten Telophase (Siehe Fig. 2j-l) auftrat, muss konkludiert werden, dass


 FIG 2. *Lancifolia*

a. Pollen in Zuckerlösungen: zwei Typen: grosser und kleiner Pollen, *b₁* und *b₂*. Diakinese mit 18 Bivalente und 5 Bivalente + 1 Univalentes (zusammen $23_{II} + 1_I$), P.M.C.; *c* und *d*. P.M.C., erste Metaphase, Polaransicht: 23 Bivalente und 1 univalentes Chromosom (heller gefärbt); *e* und *f*. P.M.C. Dasselbe wie *c* und *d*, aber in Seitenansicht; *g* und *h* P.M.C. Zweite Metaphase mit 23 und 24 Chromosomen; *i*, *k* und *l*. P.M.C. Zweite Anaphase; Nachhinken von je einem Chromosom.

diese Veränderung nicht im univalenten, sondern in einem anderen Chromosom stattgefunden hat. Die *typica*, welche aus solchen *lanceifolia*-Pflanzen entstehen, sind äusserlich in keiner Weise von den *typica* zu unterscheiden, die keine retardierende Chromosome in der zweiten Anaphase aufweisen. Hier haben wir also einen schönen Fall, bei dem Prä-Mutation sichtbar gemacht wird.

Es ist nämlich leicht vorstellbar, dass dieses Nachhinken von Zeit zu Zeit zu abnormalen Spaltungen führt, welche die Bildung neuer Mutationen mit sich bringen können (sekundäre Mutation).

§ 3. *Chlorina*-Mutanten vom Typus $2n + 1$ und $2n + 2$

In der F_1 der X-bestrahlten *typica* entstand eine Mutante, die wegen ihrer gelblich-grünen Blattfarbe als *chlorina* bezeichnet wurde. Es war eine verwickeltere Mutante, bei der ursprünglich, wie aus der Nachkommenschaft hervorging, mehrere Gen-Mutationen und wenigstens eine Chromosom-Mutation stattgefunden hatten (Typus $24_{II} + 1_I$).

Es kamen aus dieser *chlorina*- F_1 in den folgenden Generationen andere Chromosom- und Gen-Mutanten hervor; es entstanden eine grosse Menge verschiedener Formen, aus denen durch Inzucht mehrere Linien gezüchtet wurden (konstante, ziemlich konstante und immer aufspaltende Formen). In der ersten Abhandlung (3) wurde schon die *B-chlorina*, eine Gen-Mutante, besprochen.

Als Chromosom-Mutanten können wir die *A-chlorina* und ihr Derivat die *D-chlorina* erwähnen. Ihr genetisches Verhalten bei Selbstung und reziproke Kreuzung mit *typica* war so abnormal, dass es auf keine Weise möglich sein würde, diese Formen als Gen-Mutanten aufzufassen.

Es wurde nämlich die folgende eigenartige Situation bei der Spaltung gefunden (abgesehen vom Entstehen sekundärer Mutanten):

A-chlorina geselbstet gibt: *A-chlorina*, *D-chlorina*, *typica*

A-chlorina \times *typica* reziprok gibt: *A-chlorina*, *typica*

D-chlorina geselbstet gibt: *A-chlorina*, *D-chlorina*, *typica*

D-chlorina \times *typica* reziprok gibt: *A-chlorina*, *D-chlorina*, *typica*

Die *typica* aus allen Nachkommen liefert bei Selbstung nur *typica*.

Die Verhältniszahlen waren stark wechselnd. Mit Hilfe cytologi-

scher Studien konnte dieses Verhalten wie nachfolgend geklärt werden:

A-chlorina ist eine trisome Mutante (Typus $2n + 1$).

Es werden daher zwei Typen von Geschlechtszellen gebildet 24_I (normal) und $23_I + 1_{II}$. Bei Selbstung entstehen daher 24_{II} (*typica*), $23_I + 1_{III}$ (*A-chlorina*) und $23_I + 1_{IV}$, welche letzte Form lebensfähig ist und *D-chlorina* darstellt. Das Entstehen von *typica* und *A-chlorina* bei Kreuzung mit *typica* ist dann auch ohne weiteres verständlich.

Die *D-chlorina* zeigt während der Reduktionsteilungen zahlreiche Abnormalitäten. Bei der ersten Metaphase und Anaphase konnte u.a. folgendes beobachtet werden:

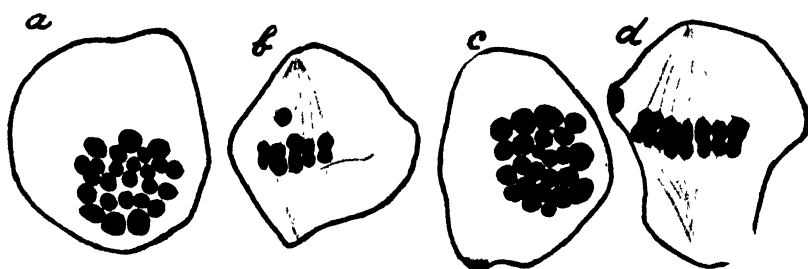


FIG 3 *D-Chlorina*, P.M.C.

a Erste Metaphase. 25 bivalente Chromosome (Polaransicht); b Erste Metaphase, Auseinandergehen eines bivalenten Chromosoms nach einem Pol; c, Erste Metaphase mit 24 bivalenten Chromosomen in der Äquatorialplatte und mit einem bivalenten exzentrischen Chromosom (Ausscheidung), Polaransicht; d Dasselbe wie c in Seitenansicht.

1. Die 25 bivalenten Chromosome (Fig. 3a) gehen während der Anaphase normal auseinander (Geschlechtszellen mit $23_I + 1_{II}$).
2. Schon während der Metaphase bewegt sich ein bivalentes Chromosom nach einem Pole (Fig. 3b) voraus, die übrigen 24 Bivalenten teilen sich nachher normal. Wir müssen uns vorstellen, dass dieses vorausseilende bivalente Chromosom eines der beiden homologen bivalenten Chromosome darstellt. Es können auf diese Weise Geschlechtszellen mit $23_I + 1_{III}$ und 24_I entstehen.
3. Oft wird auch schon während der ersten Metaphase exzentrisches Ausscheiden eines bivalenten Chromosoms wahrgenommen (Fig. 3c und d). Auch auf diese Weise können noch normale 24_I -Chromosome entstehen.

Wenn angenommen wird, dass in diesem Falle, wie auch bei anderen derartigen Trisomen und Quatrosomen oft beobachtet wurde, Formen mit mehr als 4 homologen Chromosomen nicht lebensfähig sind, so ist jetzt das genetische Aufspalten bei Selbstung und Kreuzung mit *typica* völlig zu verstehen. Es werden nämlich die Geschlechtszellen 24_I , $23_I + 1_{II}$ und $23_I + 1_{III}$ gebildet. Sowohl bei Selbstung als auch bei Kreuzung mit 24_I (*typica*) entstehen daraus 24_{II} (*typica*), $23_{II} + 1_{III}$ (*chlorina-A*) und $23_{II} + 1_{IV}$ (*chlorina-D*).

Dass bei diesen abnormalen Reduktionsteilungen in aufeinanderfolgenden Kreuzungen wechselnde Verhältniszahlen der verschiedenen Formen erhalten werden, braucht uns nicht zu wundern. Auch dass bei diesen Spaltungen die Möglichkeit zur Bildung neuer Formen (sekundäre abnormale Mutanten) besteht, kann man leicht einsehen.

Die *A-chlorina* bildet eine Analogie zu der von CLAUSEN und GOOD-SPEED (1) studierten spontanen trisomen Form „enlarged“ ($23_I + 1_{III}$) und die daraus hervorgegangenen Form „super-enlarged“ ($23_I + 1_{IV}$) ist eine Analogie zu der *D-chlorina*.

§ 4. Die Anwendung der künstlichen Mutation

Es besteht die Möglichkeit, auf diese Weise neue, für die Landwirtschaft wertvolle Formen zu erhalten. Aber dabei muss man speziell mit Chromosom-Mutanten vorsichtig sein, da daraus auch unerwünschte Formen hervorgehen können. Durch intensives Studium über diese Mutanten kann man sich gegen die unerwartete Bildung neuer unerwünschter Formen sichern. Wird überdies vor der eigentlichen Aussaat ein kleiner Teil der Saat ausgelegt, so kann man, falls dabei ein zu grosser Prozentsatz an Abnormalitäten festgestellt wird, deren ganzen Aussaat vermeiden.

Seit mehreren Jahren wird eine der erhaltenen *chlorina*-Mutanten (als F_1 -Kreuzung mit *typica*) in der Vorstenlandschen Tabakkultur wegen der schönen hellen Blattfarbe angebaut. Im Jahre 1936 waren es bereits ungefähr 10% des Gesamtareals der Tabakböden in den Vorstenlanden.

Die meisten der in dieser und in der vorigen Abhandlung beschriebenen Formen sind äusserlich vollkommen „normale“ Pflanzen. Es braucht aber nicht zu wundern, dass eben bei Derivaten von Chromosom-Mutanten dann und wann Abnormalitäten auftreten können.

Daher muss man in der Praxis bei Awnendung dieser neuen Formen speziell in den ersten Jahren darauf achten.

Ich will nun mit der Erörterung über das Auftreten solcher Abnormalitäten bei Derivaten der *chlorina* schliessen. In Fig. 5, 6 und 7 sind die Formen X-, Y- und Z-*chlorina* und deren Blätter abgebildet. Diese Formen geben bei Selbstung alle 3 Typen zurück, überdies erhalten wir noch *typica*-Formen. Obwohl die Chromosom-Analyse dieser Typen nicht abgeschlossen werden konnte, steht wohl fest, dass es sich um Typen von (X- bis Z-*chlorina*) mit immer höheren Chromosom-Zahlen ($2n + 1$, $2n + 2$ Typen usw.) handelt. Die Sterilität wird dabei immer grösser. Obwohl noch lebensfähig, hat die Zunahme der Chromosomen zur Folge, dass das Gleichgewicht in der Pflanze immer weiter zerstört wird.

Das zeigt sich hier z.B. in dem stark zugenommenen Wachstum des Mesophylls im Gegensatz zu dem der Nerven, wodurch beim Auswachsen schliesslich völlig zerrissene und deformierte Blattflächen entstehen. Die Z-*chlorina* zeigt als Folge dieser zerstörten Funktion der Blätter ein langsames Krüppelwachstum.

ZUSAMMENFASSUNG

1. Es gibt Übergänge zwischen Chromosom-Mutation und Gen-Mutation. Es wird ein Fall beschrieben, wo „Retardierung“ eines Chromosoms in der ersten Anaphase ohne Veränderung der Chromosomen-Zahl als künstliche Mutation auftrat (*oligofolia*).

2. Es wird eine künstliche ($2n - 1$)-Mutation (*lancifolia*) erhalten, die genetisch und cytologisch grosse Übereinstimmung mit der von CLAUSEN und GOODSPEED beschriebenen spontanen „fluted“-Tabakmutante zeigt.

3. *Lancifolia* spaltet *typica*-Pflanzen ab, die ein völlig normales *typica*-Aussehen haben, wobei cytologisch aber je ein Chromosoms „Retardierung“ in der späten zweiten Anaphase erkennen lässt, was die ursprüngliche *typica* nicht aufweist. Hier wird Prä-Mutation sichtbar gemacht, verursacht durch die X-Bestrahlung, was in späteren Generationen leicht zu einer sekundären Mutation ausgelöst werden könnte (sogenannte „spontane“ Mutation).

4. Das genetische Verhalten der künstlichen ($2n + 1$)-Mutation A-*chlorina* und ihres Derivats ($2n + 2$)-D-*chlorina* wird cytologisch

geklärt. Es zeigt sich Übereinstimmung mit der von CLAUSEN und GOODSPEED beschriebenen, spontanen Tabak-Mutante „enlarged“ und ihres Derivats „super-enlarged“.

5. Die Anwendung der neuen Formen in der landwirtschaftlichen Praxis ist möglich, da viele Mutanten ganz normales Aussehen haben; in den Vorstenlanden ist diese Anwendung tatsächlich schon verwirklicht. Man soll aber erst die Formen gut studieren und Massnahmen treffen, um das unerwünschte Auftreten von abnormalen Derivaten rechtzeitig zu entdecken. Abnormalitäten sind nämlich speziell bei abnormalen Chromosomzahlen zu erwarten und kommen auch wirklich vor (*X*-, *Y*-, *Z-chlorina*).

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Fig. 4 Die *Lanceifolia*-Mutation links (hoher, mehr und schmalere Blätter, Früchte spitziger) neben die *typica* rechts



Fig. 5 *a. X-chlorina*, Blattlamina zu groß für den Blattrand,
b. Y-chlorina, die gleiche Eigenschaft noch stärker entwickelt, wodurch
 die Blätter beim Alterwerden Locher bekommen.



Fig. 6 *Zehlniana*, die gleiche Eigen-schaft in besonders stärker Ausprägung, wodurch die ganze Pflanze deformierte, zerrissene Blätter erhält.



Fig. 7 Ausgewachsene Blätter von *a* *X-chlorina* *b* *Y-chlorina* und *c* *Z-chlorina*.

EXPERIMENTAL PRODUCTION OF HAPLOIDS IN NICOTIANA RUSTICA L.¹⁾ (AND A DISCUSSION OF HAPLOIDY IN FLOWERING PLANTS)

by

M. A. IVANOV

Laboratory of Plant Genetics, Leningrad State University, Leningrad,
U.S.S.R.

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¹⁾ Translated from the Russian by E. BRISSENDEN.

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INTRODUCTION

Prior to the present investigation haploid plants had been obtained in the following species of *Nicotiana*: *N. tabacum*, *N. glutinosa*, *N. sylvestris* and *N. Langsdorffii*. All of the haploids in these species arose as single individuals in large progenies from distant crosses. Thus, haploid *tabacum* was first obtained in 1924, *i.e.*, only two years after the first haploid sporophyte in the higher flowering plants had been reported — haploid *Datura stramonium* (7). Two haploid *tabacum* plants appeared in the F_1 from the cross *N. tabacum* var. *purpurea* \times

N. sylvestris (15, 12). The second case of haploid *tabacum* was reported in 1928 (68), when five haploid *tabacum* plants appeared in the F_1 from crossing this species with *N. sylvestris* and *N. tomentosa*.

The third instance of haploid *tabacum* occurred as a result of crossing the synthetic species *N. digluta*, having 24 bivalent chromosomes from *N. tabacum* and 12 bivalent chromosomes from *N. glutinosa*, with diploid *N. tabacum*, the latter being used as the pollen parent (14). The investigators reporting this haploid regard it as a case of haploid merogony. A single haploid plant was obtained in a population of 173 F_1 individuals. The same year (1929) KHRISTOV (39) found a *tabacum* haploid, which had arisen spontaneously in the progeny of normal, self-pollinated *tabacum* plants. The fifth case of haploid *tabacum* was reported by MCCRAY in 1932. This haploid was found among F_1 plants from the cross *N. tabacum* var. *angustifolia* ♀ × *N. glutinosa* ♂.

Lastly, in 1935, *tabacum* haploids were obtained by S. P. HACHATUROV, P. A. POVOLOCHKO, and M. F. TERNOVSKY. The first-mentioned investigator obtained one haploid from the cross *N. tabacum* ♀ × *N. quadrivalvis* ♂ (25); the second — one haploid by crossing *N. tabacum* ♀ and *N. alata* ♂, the flowers after pollination being subjected for 24 hours to low temperature (+ 3° C.), and three haploids by crossing *N. tabacum* and *N. Rusbyi* and using high temperature (+ 40° — + 45° C.) for 4-5 hours after pollination (64). TERNOVSKY found one *tabacum* haploid among F_1 *tabacum-longiflora* and another among F_1 *tabacum-glutinosa* plants (76).

Of the three *N. glutinosa* haploids one arose spontaneously in field cultures, and two were found in the F_3 from intercrossing *N. glutinosa* plants grown from X-rayed seed and characterized by low vigor and fertility (23, 80). The appearance of the latter two haploids is considered by WEBBER to have been due to parthenogenesis stimulated by the high degree of incompatibility of the male and female gametes.

Haploid plants of *N. sylvestris* and *N. Langsdorffii* have been reported by D. KOSTOFF (42, 43). In both cases the haploids were obtained from interspecific crosses as a result of the development of an embryo from a male generative cell (androgenesis). The *Langsdorffii* haploid was obtained by pollinating a *tabacum* triploid ($2n=72$) with *Langsdorffii* ($2n=18$) pollen; the *sylvestris* haploid

by pollinating F_1 *tabacum-sylvestris* hybrids with *sylvestris* pollen.

The great majority of haploids in other families, genera, and species of the higher plants originated in similar ways. They arose either as a result of distant hybridization or spontaneously, without apparent cause, in pure lines or in the progeny of interracial hybrids.

Most of the haploid plants so far reported occurred accidentally, and only a few as the result of specific, active interference on the part of the investigator. Some of the haploid plants of *Zea Mays* (71), *Triticum monococcum* (35), *Tr. dicoccum* and *Tr. persicum* (84) were obtained as the result of pollination with X-rayed pollen, and some haploids of *Datura stramonium* (7) and *N. tabacum* (64) appeared after subjecting plants at time of fertilization to low or high temperature.

Since obtaining haploid plants not only is of great theoretical interest but also may prove to be of practical importance as a means of securing absolutely homozygous forms (EAST, KARPECHENKO, NAVASHIN), their artificial production, as the result of active interference on the part of the investigator, acquires exceptional interest.

In 1934, when the importance of this problem had just begun to be realized, Professor G. D. KARPECHENKO suggested that I take as the theme of my work the experimental production of haploids in some species of *Nicotiana*. The species selected was *N. rustica*, and several methods of stimulating egg-cells to parthenogenetic development were tested, namely: (1) puncturing ovaries of emasculated flowers with needles to test the effect of wound hormones; (2) removing the style shortly after pollination to test the effect of pollen tubes during their growth in the style; (3) pollination with pollen from plants belonging to the same family but not closely related to *Nicotiana*; and (4) pollination with X-rayed pollen from the same species.

In addition, as thorough a morpho-cytological analysis as possible was made of the *N. rustica* haploids obtained.

PUNCTURING OVARIES OF EMASCULATED FLOWERS

In order to find out if parthenogenesis might be artificially induced by puncturing the tissues surrounding the ovules, *i.e.*, by calling into action wound hormones (HABERLANDT, 1922), we emasculated with particular care 150 flowers of *N. rustica* var. *humilis* and 120 flowers

of *N. rustica* var. *texana*. The emasculated flowers were at once bagged to protect them from pollination. The punctures were made with a very finely pointed glass needle, similar to those of a microdissection apparatus. Half of the emasculated flowers were punctured on the second day and the other half on the fourth day after emasculation. The number of punctures given each ovary varied from 5 to 50, the maximum being given in but one case.

It was observed that during the first few days after the puncturing the ovaries began to develop very rapidly, particularly the placental and other inner tissues. Forty hours after the puncturing the ovaries already had a diameter of 5 millimeters, increasing to 7 mm. by the fifth day, while the control flowers (emasculated but not pollinated nor punctured) had by that time all dropped off, the diameter of their ovaries not being over 2-3 mm. Most of the punctured ovaries, due to the fact that their walls (carpels) did not grow as rapidly as the placental tissues, cracked along the lines marked by the punctures, and in places small ovules were revealed.

Shedding of the ovaries began on the 10th-12th day after puncturing, and within the course of two or three days all had dropped off. Every one was picked up and examined, and not a single seed, not even a puny one, was found in all the 270 ovaries.

Cytological investigation of the ovaries, fixed on the eighth day after puncturing, clearly showed that the induced development affected only the ovary walls, the placentae, and the integuments of a few ovules, while the embryo sacs remained in the same condition as usual prior to fertilization, except that most of them showed signs of extreme degeneration. At this time the embryo sacs of normally fertilized *N. rustica* plants already had well-developed, multicellular endosperm and quite well-formed embryos.

As a result of this experiment it seems that puncturing the ovaries of emasculated flowers in *N. rustica* induces very rapid development of the somatic tissues comparable to that resulting from normal fertilization. However, development of the generative parts of the ovules apparently cannot be stimulated in *N. rustica* by this method, or, if possible, the chances of success are exceedingly small. We did not obtain a single seed, despite the fact that we punctured 270 ovaries, each of which contained more than 600 ovules, *i.e.*, we did not have a single success out of 162,000 chances.

TESTING THE POSSIBILITY OF POLLEN TUBES HAVING A STIMULATIVE EFFECT

Of great interest is the problem as to whether it is possible for growing pollen tubes to stimulate egg-cells to parthenogenetic development. The assumption is that the ability to stimulate is an attribute not only of the sperm nucleus (generative cell) which has penetrated the embryo sac but also of the pollen tube growing in the style. To carry out experiments capable of definitely deciding this question is exceedingly difficult, but some data as to the possibility of pollen tubes having a stimulative effect during the period of their growth in the style was obtained by removing the style prior to the tubes reaching the embryo sac, and testing whether any effect had been produced.

In these experiments we pollinated emasculated flowers with normal pollen, the exact time of pollination being noted in each case. After pollination the flowers were immediately bagged again. Then, beginning five hours later and at stated intervals the styles of some of the flowers were removed by plucking them off close to the ovary, thereby removing the growing pollen tubes also.

In order to facilitate observations in the subsequent generation, we used pollen from plants of the variety *texana*, which has broad leaves (a dominant character), while as mother plants we selected plants of the variety *humilis*, having narrow leaves (a recessive character). This made it very easy to determine the origin of each F_1 plant, i.e., to decide whether it arose as a result of parthenogenesis or normal fertilization.

As controls for this experiment we used two sets of flowers — 50 in each set. The first set of control flowers were emasculated but not pollinated. In their case, after four of five days, the flowers dropped off, together with their stems, without the ovaries having shown any perceptible increase in size. The second set of control flowers, of the variety *humilis*, were emasculated and then pollinated with *texana* pollen, the style not being removed. These flowers all produced normally developed capsules full of seed.

The length of time the pollen tubes were allowed to grow, the number of flowers pollinated, and the results obtained in this experiment are given in Table 1 below.

TABLE 1. FORMATION OF CAPSULES AND SEEDS BY FLOWERS WHOSE STYLES WERE REMOVED AT DIFFERENT LENGTHS OF TIME AFTER POLLINATION

No of hours between pollination and removal of style	First replication				Second replication			
	Flowers pollinated	Capsules obtained	Seeds obtained	Average no of seeds per capsule	Flowers pollinated	Capsules obtained	Seeds obtained	Average no. of seeds per capsule
5.0	15	0	—	—	20	0	—	—
6.0	15	0	—	—	19	0	—	—
8.0	15	0	—	—	18	0	—	—
8.5	17	0	—	—	20	0	—	—
9.0	15	0	—	—	20	0	—	—
9.5	14	2	14	7.0	20	2	12	6.0
10.0	24	4	75	18.7	18	2	37	18.5
10.5	19	8	133	16.6	17	4	63	15.8
11.0	16	8	198	24.8	—	—	—	—
11.5	15	15	585	39.0	16	13	437	33.6
12.0	11	11	336	30.5	—	—	—	—
13.0	14	14	1386	99.0	—	—	—	—
18.0	11	11	5079	461.7	—	—	—	—
Control	Emasculated but not pollinated				Emasculated and pollinated			
	50	0	—	—	50	50	23,635	472.7

As is seen from Table 1, the effect of pollen tubes which have grown in the style for from five to nine hours is insufficient to produce either the assumed stimulation or fertilization, for all the ovaries without exception dropped off during the first few days, closely resembling in this respect the control flowers which were not pollinated. When the pollen tubes were allowed to grow for 9.5 hours, a few seed were formed in occasional capsules. Thus, in the first replication there were obtained from 14 pollinated flowers 2 mature capsules, averaging 7 well-developed seeds each; in the second — from 20 flowers 2 capsules, averaging 6 well-developed seeds each. As the

time the pollen tubes were allowed to grow was lengthened, the number of seeds obtained steadily increased, until, when the pollen tubes were permitted to grow for 18 hours, it became fully normal.

The seeds obtained were sown the following year and germinated very well. All the seedlings without exception were so very markedly hybrid in character that it was not possible to suspect even a single plant of having originated by generative or somatic parthenogenesis.

A cytological investigation of the styles, fixed at the time of removal, gave an idea as to the rate of pollen-tube growth within the style. The pollen tubes begin to grow 20–25 minutes after pollination. Five hours later the tubes penetrate the style, having passed through the stigma but as yet only slightly beyond it. Nine hours after pollination the ends of a few of the tubes are found at the base of the style, but most of them are still in the mid-region. A small number of microspores, even nine hours after being placed on the stigma, have either only just begun to germinate or have very short tubes. In styles removed ten hours after pollination most of the tubes still remained within the limits of the style, only a few, occasional ones being broken in two when the style was removed from the ovary.

This cytological analysis agrees well with the data in Table 1. On the basis of this experiment and the cytological analysis we believe that the following conclusions may be drawn: (1) In *Nicotiana rustica* pollen tubes growing in the style for nine hours have no stimulating effect, or it is insufficient to induce the generative parts of the embryo sac to develop parthenogenetically. (2) After the pollen tubes have grown in the style for more than nine hours, a few cases of fertilization are observed. (3) By this time some of the pollen tubes have reached the entrances to the embryo sacs, but even here, apparently, they produce no stimulating effect.

POLLINATING *Nicotiana rustica* WITH POLLEN FROM PLANTS BELONGING TO OTHER GENERA OF THE *Solanaceae*

As we have already mentioned, most of the haploid plants obtained by various investigators have occurred as the result of interspecific and intergeneric crosses. We did not undertake to conduct extensive hybridization experiments with *Nicotiana rustica*. In the few crosses which we did make the following were used as the pollen plants:

1. *Solanum nigrum* L.
2. *Lycopersicum esculentum* MILL.
3. *Petunia violacea* LINDL.
4. *Datura stramonium* L.
5. *Atropa belladonna* L.

In each combination the number of flowers pollinated was 30. As a result, seeds were obtained in only two cases: 20 small, shriveled seed from the cross *Nicotiana rustica* ♀ × *Atropa belladonna* ♂ and one well-formed seed from the cross with *Lycopersicum esculentum* (tomato). In the case of all the other combinations we did not succeed in obtaining any seed.

The twenty seeds obtained from pollinating *Nicotiana rustica* flowers with *Atropa* pollen failed to germinate. The one seed obtained from pollination with tomato pollen, however, produced a normal seedling, which developed into a healthy plant, indistinguishable as regards all morphological characters from a normal diploid *N. rustica* plant. This plant was normally fertile, and had in its somatic cells the diploid number of chromosomes (48) possessed by *N. rustica*. Such a purely maternal plant could arise only as the result either (1) of somatic parthenogenesis (or, perhaps, generative parthenogenesis with subsequent doubling of the chromosome number) or (2) of simple "self-contamination" at the time of emasculation.

In the summer of 1934, in carrying out all these experiments there were emasculated, including the controls, a total of about 1,500 flowers, and not in a single case did we have the slightest indication of "self-contamination" at the time of emasculation. This alone makes contamination in the case here under consideration very improbable, since equally great care as regards emasculation was taken in all experiments, and all emasculation operations were performed by the writer personally. The probability of contamination at the time of emasculation becomes still smaller, if it is taken into consideration that in this particular case contamination would have had to occur in one single bud and by one single microspore. It would, of course, be far more probable to expect contamination by many microspores simultaneously, but this would have resulted in the development of several seeds in the capsule.

Taking all this into account, we were convinced that this was a case either of somatic parthenogenesis or of generative parthenogenesis

followed by doubling of the chromosome number in the early stages of embryonic development. In our subsequent experiments we sought for similar cases, and encountered them among the progeny from flowers pollinated with X-rayed pollen, which further confirmed the possibility of the occurrence of diploid parthenogenesis in *Nicotiana rustica*.

In our opinion the foregoing suffices to establish the parthenogenetic origin of the plant obtained from the cross *N. rustica* var. *texana* ♀ × *Lycopersicum esculentum* ♂.

POLLINATION WITH X-RAYED POLLEN

The method of pollination with X-rayed pollen was used in the reciprocal crosses: *N. rustica* var. *humilis* ♀ × *N. rustica* var. *texana* ♂ and *N. rustica* var. *texana* ♀ × *N. rustica* var. *humilis* ♂. Prior to pollination mature pollen was X-rayed and at once placed on the stigma of flowers which had been emasculated two or three days previously. Only the pollen was subjected to the X-ray treatment; the female generative organs were not given any treatment. The pollen was taken from flowers which had just opened and begun to shed their pollen. Such flowers were plucked and placed under an X-ray lamp. In each case an abundance of X-rayed pollen was placed on the stigma. Immediately after pollination each flower was labelled and bagged. Later the seed of each individual capsule was gathered and analyzed separately, and the following year the progeny of each capsule was grown separately under its respective label.

The conditions of X-radiation, the number of flowers pollinated with the X-rayed pollen, and the number of capsules set depending on the X-ray dosage are given in Table 2 (page 305).

The effect of X-rays on the fertilizing capacity of male gametes

It was to be expected, first of all, that, as the X-ray dosage was increased, the microspores would gradually lose their germinating capacity. In order to elucidate this matter, the pollen subjected to the various X-ray doses was at once placed in Petri dishes filled with an artificial germinating medium (1% agar-agar + 5% sugar). At the same time, by way of control, normal, non-irradiated pollen was similarly tested for germination.

TABLE 2. NUMBER AND PERCENTAGE OF CAPSULES SET DEPENDING ON X-RAY DOSAGE GIVEN POLLEN OF *N. rustica* VAR. *texana* USED TO POLLINATE *humilis* FLOWERS

(General conditions of irradiations: 120 kV, 5 mA, filter 1 m/m Al.)

X-ray dosage (in r units)	Irradiation conditions			Flowers pollinated	Capsules set	Percentage of capsules set	
	Target distance	Exposure					
		hr	min	sec			
4,000	30 cm.	1	6	40	32	31	97.0
6,000	"	1	40	00	24	24	100.0
8,000	"	2	13	20	19	19	100.0
10,000	"	2	46	40	25	25	100.0
12,000	"	3	20	00	30	30	100.0
15,000	"	4	10	00	30	27	90.0
17,000	"	4	43	20	26	23	88.5
19,000	"	5	16	40	37	30	81.1
21,000	"	5	50	00	31	27	87.1
23,000	"	6	23	20	35	30	85.7
26,000	"	7	13	20	34	23	67.6
28,000	"	7	46	40	28	11	39.3
30,000	"	8	20	00	35	18	51.4
32,000	"	8	53	20	35	20	57.2
34,000	"	9	26	40	19	12	63.2
35,000	16.5 cm.	3	00	3	20	10	50.0
40,000	"	3	25	46	25	11	44.0
45,000	"	3	51	29	30	12	40.0
50,000	"	4	17	12	40	19	47.5
55,000	"	4	42	55	53	21	39.6
60,000	"	5	8	38	49	19	38.8
65,000	"	5	34	21	35	12	34.3
70,000	"	6	00	5	30	8	26.7

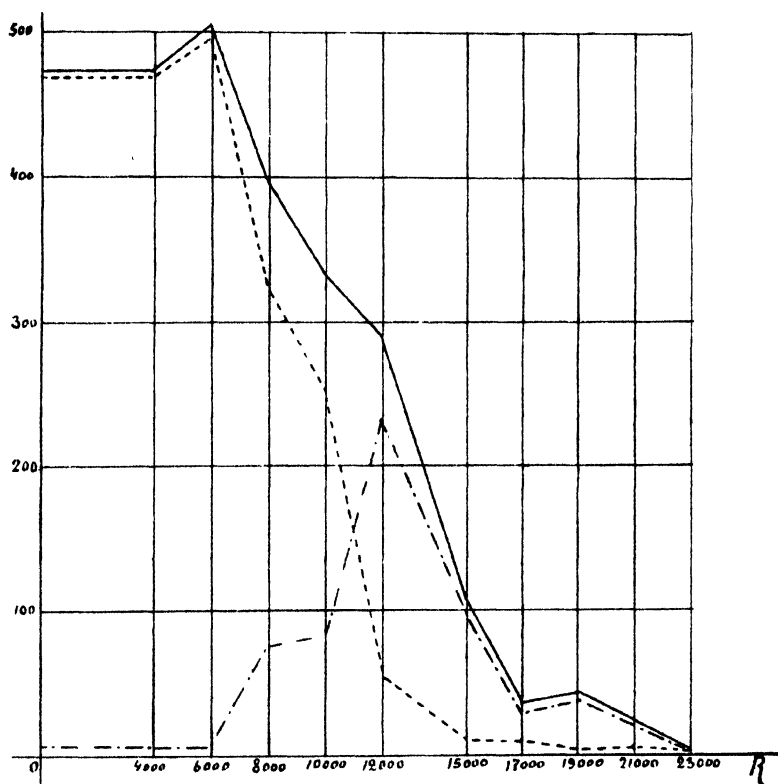
The non-irradiated pollen began to germinate within 20–25 minutes after sowing, and division of the generative nucleus was observed after 18–20 hours. Pollen grains treated with comparatively small X-ray doses (4,000–6,000 r) began to germinate even somewhat

sooner, and the growth of their tubes was more rapid. As the dosage was increased there was observed at first only an inconsiderable decrease in the energy of germination of the pollen, which varied but slightly from that of the control. Such an inconsiderable decrease was observed in the case of X-ray doses of from 12,000 to 30,000 *r*. Further increase of the dosage, however, resulted in a marked decrease in the energy and uniformity of germination, some of the pollen grains (as many as 20%) not having germinated even after 24 hours. Unfortunately, we did not succeed in attaining an X-ray dosage heavy enough to stop completely pollen-tube growth of *N. rustica* pollen, if this is possible. At any rate, a dosage of 70,000 *r* is not sufficient. Pollen-tube growth persisted even at this dosage, the percentage of microspores not germinating after 24 hours not exceeding 20–25

Special note should be taken of the remarkable resistance of *N. rustica* pollen to the action of X-rays. It is true, as will be shown further on, that considerably smaller doses are required to disturb the regular course of division of the generative nuclei than to lower the energy of pollen-tube growth, but even these are very large in comparison with those reported as being close to lethal in the case of wheat pollen, *viz.*, 2,500–3,000 *r* (35 and 84). In our case, as we have shown, even 70,000 *r* is not a lethal dose for *N. rustica* pollen, although completely sterilizing it.

A detailed cytological study of the behavior of the pollen tubes after irradiation was not made. For this a special investigation is needed. However, a few preliminary observations were made by the aceto-carmin method. It was noted that X-radiation begins to have a marked effect on division of the generative nucleus at a dosage of 15,000 *r*. Division often proceeded so irregularly that, as a result, there could clearly be seen in some pollen tubes --- instead of two generative nuclei as normally --- three or four nuclei, or rather fragments of nuclei, of unequal size. Such nuclei, apparently, cannot be normally functional, since they do not contain a full set of chromosomes. These preliminary cytological observations fully correspond to the data obtained by PODDUBNAJA-ARNOLDI from a detailed study of the pollen tubes of X-rayed pea pollen (63).

Such an effect of X-radiation on the formation of the male generative nuclei cannot help but be reflected in the quantity and quality of the seeds obtained from pollination with X-rayed pollen and also



GRAPH 1 Graph showing relation between number of seeds obtained per capsule in *N. rustica* and X-ray dosage given pollen.

in the morphology and viability of the plants grown from these seeds. The average number of seeds obtained per capsule by the use of pollen subjected to different X-ray doses is given in Table 3. For the sake of a more vivid portrayal the data in Table 3 are also presented in the form of a graph.

TABLE 3. NUMBER OF SEEDS OBTAINED PER CAPSULE IN *Nicotiana rustica* BY THE USE OF X-RAYED POLLEN

X-ray dosage (in units)	Number of capsules	Plump, normal-appearing seeds			Shriveled seeds			Average no of seeds per capsule	Shriveled seeds (in %)
		Average no of seeds per capsule ($M \pm m$)	$M_1 - M_2$	$\sqrt{m_1^2 + m_2^2}$	Average no of seeds per capsule ($M \pm m$)	$M_1 - M_2$	$\sqrt{m_1^2 + m_2^2}$		
Without irradiation	10	466.16 \pm 16.60			4.10 \pm 0.85			470.26	0.9
4,000	17	465.97 \pm 17.60	0.01		3.85 \pm 0.64	0.24		469.82	0.8
6,000	19	498.98 \pm 15.93	1.40		5.15 \pm 0.79	1.29		504.13	1.0
8,000	16	324.50 \pm 17.45	7.40		75.03 \pm 7.59	9.16		399.53	18.7
10,000	20	253.50 \pm 17.88	2.80		81.64 \pm 5.60	0.70		335.14	24.4
12,000	10	55.50 \pm 8.49	10.01		235.50 \pm 10.22	13.21		291.00	80.9
15,000	18	9.17 \pm 1.34	5.39		97.83 \pm 10.86	9.23		107.00	91.5
17,000	18	8.94 \pm 1.29	0.12		26.38 \pm 1.32	6.53		35.32	(74.0)
19,000	13	2.18 \pm 0.51	4.85		39.12 \pm 9.93	1.27		41.30	(94.9)
21,000	20	3.15 \pm 0.80	1.02		19.80 \pm 2.32	18.9		22.95	(86.5)
23,000	25	0.20 \pm 0.11	3.64		0.35 \pm 1.71	6.75		0.55	()
Over 23,000	--	No seeds obtained							

The distribution of the seeds into groups according to degree of plumpness and the counting of the seeds were done with the aid of a large magnifying glass. In the table we have given data for only two groups — "plump, normal-appearing" and "shriveled" (see Phot. 1) — separately and combined. But, in addition to these two categories, there were in each capsule many seeds which we designated "very shriveled". They were flat and greatly shriveled and varied so much in size that it was very difficult to distinguish between them and undeveloped, dried up ovules.

The data in Table 3 give a very good picture of the sterilizing effect

of the X-ray doses on the microspores of *N. rustica*, since the number of seeds per capsule and their quality represent in this case the result of the functioning of the generative nuclei of the pollen used.

The total number of seeds (plump, normal-appearing plus shriveled) obtained per capsule, when non-irradiated pollen was used, amounted to 470.26. Approximately the same number was obtained when the pollen was given X-ray doses of 4,000 or 6,000 *r*. The slight difference recorded lies within the limits of experimental error. In both cases the seeds were practically all of normal appearance, the average number of shriveled seeds not exceeding 5.15 ± 0.79 . Here the differences are likewise within the limits of error. When the X-ray dosage was raised to 8,000 *r*, there was observed a slight decrease in the total number of seeds per capsule (399.5), while the number of shriveled seeds showed a sharp increase — from 5.15 ± 0.79 at 6,000 *r* to 75.03 ± 7.59 at 8,000 *r*. With further increase of the X-ray dosage the total number of seeds per capsule rapidly decreased, dropping at 17,000 *r* to only 35, most of which were shriveled (26 out of 35). When the pollen was subjected to an X-ray dose of 23,000 *r*, we obtained only occasional capsules with from 1 to 3 seeds. The average number per capsule was 0.55. Higher doses completely sterilized the pollen, and no seeds were obtained, neither normal nor shriveled, nor even "very shriveled". The capsules contained only undeveloped, dried up ovules.

All the foregoing relates to the combination *N. rustica* var. *humilis* \times *N. rustica* var. *texana* δ , i.e., to the sterilizing effect of X-rays on the pollen of the variety *texana*. In the case of the reciprocal cross, where the pollen of the variety *humilis* was subjected to X-radiation, the only difference revealed was a somewhat greater resistance of the pollen. Occasional capsules with a few seeds were obtained even at an X-ray dosage of 30,000 *r*.

Consequently, on the basis of the data obtained and presented in Table 3, we may summarize the effect of X-radiation of pollen on the seed obtained from normal flowers pollinated with such pollen as follows:

1. X-ray doses smaller than 8,000 *r* do not affect either the normality of the seeds or the quantity per capsule. There is, perhaps, even a slight stimulation of seed production, but this increase may be within the limits of experimental error.

2. Decrease in the total number of seeds per capsule — and also decrease in the number of plump, normal-appearing seeds and increase in the number of shriveled seeds — commences at a dose of 8,000 r .

3. With a further increase in the X-ray dosage, the decrease in the number of plump, normal-appearing seeds per capsule is considerably more rapid than that in their total number, a result of two trends — decrease in total number and increase in number of shriveled seeds.

4. The number of shriveled seeds per capsule increases (with a simultaneous decrease in the total number), attaining a maximum at a dosage of 12,000 r , after which, with a further increase in the dosage, there is observed a rapid drop in the number of shriveled seed.

5. The pollen of *N. rustica* var. *texana* was completely sterilized by X-ray doses above 23,000 r ; that of the variety *humilis* by doses above 30,000 r .

The germinating capacity of the seeds obtained and the percentage of seedlings surviving also proved to vary with the X-ray dosage given the pollen. When the dosage reached 12,000 r , we began to make observations as to the germinating capacity of the seeds and the viability and morphology of the plants, since beginning with this dosage, which materially reduces the set of seed, the occurrence of haploid plants may be expected. In the case of smaller doses, where the set of seed is almost normal, there is slight prospect of haploid plants occurring, since such doses apparently do not greatly affect the fertilizing capacity of the generative nuclei.

In 1935 data on seed germination were obtained incidentally in the regular course of raising the seedlings (Table 4). This was readily done, since the seeds of each capsule — divided into two groups: (1) normal-appearing plus shriveled and (2) very shriveled — were sown in separate paper boxes filled with sterilized sand.

The germinating capacity of seed obtained by the use of X-rayed pollen was, in general, very low as compared with that of control seeds (harvest of 1934), which amounted under similar conditions to 92.5 per cent. Moreover, there was a very marked tendency of the germinating capacity to decrease with the increase in the X-ray dosage. Thus, if at a dosage of 12,000 r the germinating capacity of the seeds in sand was 28.08 per cent, at 17,000 r it was 12.84 per cent and at 21,000 r — only 4.31 per cent.

In the spring of 1936 the germinating capacity of the seeds was

TABLE 4. GERMINATING CAPACITY OF SEEDS OBTAINED BY THE USE OF X-RAYED POLLEN (TESTED BY SOWINGS IN SAND, 1935)

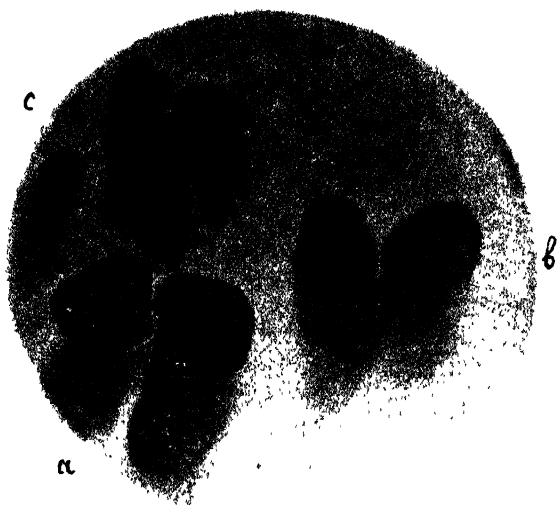
X-ray dosage (mr units)	Normal-appearing plus shriveled seeds			Very shriveled seeds		
	Seeds sown	Seedlings obtained	Germination %	Seeds sown	Seedlings obtained	Germination %
Control	200	185	92.5	—	—	—
12,000	812	228	28.08	461	6	1.30
15,000	883	218	24.69	2734	97	3.55
17,000	366	47	12.84	5012	15	0.30
19,000	447	48	10.74	2248	3	0.13
21,000	209	9	4.31	1089	1	0.09
23,000	12	2	—	100	0	—

again tested, but this time the seeds were sown in Petri dishes on filter paper in a thermostat at a temperature of 25°–27° C. Normal seeds, used as control, in this case also showed a high germinating capacity, very close to that in the former test (94.5 per cent). The seeds obtained by the use of X-rayed pollen, however, showed quite different germination percentages than those recorded in the former test, nevertheless, they preserved the general tendency to a decrease in germinating capacity as the X-ray dosage given the microspores was increased (see Table 5, page 312). This difference in the germination percentages of seeds obtained by the use of pollen given the same X-ray doses but tested in sand and on filter paper is not accidental. It is due to the low vigor and energy of growth of the young seedlings, of which many were so weak and undifferentiated in structure that they were unable to penetrate the shallow layer of sand and come to the surface. This was further confirmed by the fact that, upon transplanting all the seeds that had germinated on the filter paper to flats filled with light sandy loam, many of them, despite painstaking care, perished before making their way to the surface of the soil. The percentage of seedlings breaking through to the soil surface closely approximated the percentage of germination of the seed as previously tested in sand (1935).

In studying the germinating capacity of the seed, it was found that

TABLE 5. GERMINATING CAPACITY OF SEEDS OBTAINED BY THE USE OF X-RAYED POLLEN (TESTED BY SOWINGS
IN PETRI DISHES ON FILTER PAPER, 1936)

X-ray dosage (in r units)	Normal-appearing seeds			Shrivelled seeds			Very shrivelled seeds			Normal-appearing plus shrivelled seeds			Ability of germinated seeds to break through to soil surface	
	Seeds sown	Seedlings obtained	Germination %	Seeds sown	Seedlings obtained	Germination %	Seeds sown	Seedlings obtained	Germination %	Seeds sown	Seedlings obtained	Germination %	No. breaking through	In %
Control	200	189	94.5	—	—	—	—	—	—	—	—	—	—	—
15,000	85	72	84.7	521	318	61.04	1460	111	7.60	606	390	64.36	209	34.49
17,000	52	32	61.5	382	138	36.13	2888	40	1.38	434	170	39.17	101	23.27
19,000	5	2	—	72	5	6.94	1574	0	—	77	7	9.09	2	2.6
21,000	1	0	—	46	4	8.69	180	3	1.67	47	4	8.51	2	—
23,000	0	—	—	3	0	—	95	0	—	3	0	—	0	—



PHOT. 1. *Nicotiana rustica* seeds obtained by the use of X-rayed pollen. — *a*, plump, normal-appearing seeds; *b*, shriveled seeds, *c*, very shriveled seeds. $\times c. 200$



PHOT. 2. *N. rustica* seedlings. — Normal seedling on second day after coming up. $\times c. 200$



PHOT. 3.



PHOT. 3 and 4. *N. rustica* seedlings: Seedlings from very shriveled seed, obtained by use of X-rayed pollen, on the sixth and ninth day after coming up. Photographed under a binocular. $\times c. 200$.

the seeds which we had classed as "very shriveled" were capable of germination. This was quite unexpected, since these seeds were so shriveled that the term "seeds" could scarcely be applied to them. Most of them looked more like dried-up ovules.

The germinating capacity of the seeds of this group, as may be seen from Tables 4 and 5, was, of course, low, but quite sufficient to attract attention. The general tendency of a decrease in the germinating capacity with increase in the X-ray dosage, so marked in the group of normal-appearing and shriveled seeds, was not patent in this group. The germinating capacity of the very shriveled seeds was highest, when the pollen used had been given an X-ray dosage of 15,000 *r*. At this dosage the germination percentage of the seeds in sand was 3.55 and on filter paper even 7.6. Other X-ray doses resulted in a smaller percentage of germination of these "very shriveled" seeds (from 0.09 to 1.3 per cent, when tested in sand).

This germination of extremely undeveloped seeds was of particular interest, because among the seedlings obtained, most of which were greatly deformed, there were found a few which closely resembled the maternal plant, possessed normal vigor and fertility, and had 48 as their chromosome number. In the case of the seeds producing these seedlings there was evidently a comparatively normal development of the embryo and an entirely abnormal development of the endosperm, the latter causing the intense shriveledness.

The ability to survive of seedlings from seeds obtained by the use of X-rayed pollen is, in general, very slight, and particularly so in the case of those seedlings coming from very shriveled seed. Most of these frail seedlings, even if they succeed in reaching the surface of the soil, perish within a few days; some, however, may survive somewhat longer (10-15 days). Very often these seedlings are not able to cast off the seed coat, which adheres to them until the last. Attracted by this phenomenon, we examined with the aid of a binocular a considerable number of such seedlings, and found an interesting abnormality. Thus, if in normal plants the seed coats are cast off by well-developed, fairly broad cotyledons within the first two days after coming up, the seedlings from very shriveled seeds did not cast off the seed coats even after 10-12 days, and possessed extremely undeveloped, often deformed cotyledons. Sometimes the cotyledons were lacking altogether, and the stem terminated in a rounded cone (Phot. 2, 3 and 4).

Abnormality in structure of the seedlings obtained by the use of X-rayed pollen was also expressed in a varying number of cotyledons. There were seedlings with one, two, three, and even four cotyledons. The frequency of occurrence of these deviations from the normal type (two cotyledons) is shown in Table 6.

TABLE 6. NUMBER OF COTYLEDONS OF SEEDLINGS OBTAINED BY THE USE OF X-RAYED POLLEN

X-ray dosage (in r units)	Number of seedlings										Total
	With one cotyledon	In %	With two cotyledons	In %	With three cotyledons	In %	With four cotyledons	In %	Not shedding seed coats	In %	
12,000	15	6.61	175	77.09	32	14.10	1	0.44	4	1.76	227
15,000	9	2.94	226	73.86	34	11.11	1		37	12.09	306
17,000	3	4.92	31	50.82	3	4.92	1	1.64	23	37.70	61
19,000	5	10.00	25	50.00	5	10.00			15	30.00	50
21,000	2	(22.22)	4	(44.45)					3	33.33	9
23,000	-	-	2	-							2

The seedlings from normal-appearing and shriveled seeds possessed a greater ability to survive, but, nevertheless, the mortality rate was very high, showing a clear tendency to increase with the increase in the X-ray dosage given the pollen.

In Table 7 are given data on the mortality rate of plants during the first two months of life, *i.e.*, in the seedling stage, prior to setting out in the field, and also during the entire summer after setting out in the field. As may be seen from table, plants perished throughout the entire course of the vegetative period, but the mortality rate was considerably greater during the seedling stage. In the latter stage the effect of the size of the X-ray dose was clearly evident. Thus, if at a dosage of 12,000 r the mortality rate of seedlings was 15.81 per cent, at a dosage of 17,000 r it was 46.77 per cent, and continued to increase with increased dosage. In the more mature stage, growing in the field, the mortality rate did not show an increase with increased X-ray dosage, but was nevertheless quite high (over 20 per cent).

TABLE 7. MORTALITY RATE OF PLANTS OBTAINED BY THE USE OF X-RAYED POLLEN

X-ray dosage (in r units)	Total no of seedlings	No. of plants perishing prior to setting out in field	Mortality rate of seedlings	No of plants perishing in the field (during the summer)	Mortality rate of field plants	Total no of plants perishing	Mortality rate for entire vegetative period
12,000	234	37	15.81	64	27.35	101	43.16
15,000	315	87	27.62	62	19.68	149	47.30
17,000	62	29	46.77	16	25.81	45	72.58
19,000	51	21	41.18	14	27.45	35	68.63
21,000	10	6	(60.00)	2	20.00	8	(80.00)
23,000	2	0	—	0	—	0	—

The surviving plants were very diverse as regards morphology and fertility. A brief morphological description of them will be useful, in order to make clear the distinguishing characteristics of that group of plants among which the haploids were found

Morphological description of the plants obtained by pollinating normal pistils with X-rayed pollen

Despite the great diversity of morphological characters possessed by the plants obtained by the use of pollen treated with heavy doses of X-rays, all the plants from the combination *N. rustica* var. *humilis* ♀ × *N. rustica* var. *texana* ♂ may be readily grouped according to the following three types: (1) hybrid type; (2) aberrant type; and (3) maternal type. The last group, as the one of most interest for our purposes, may be subdivided according to degree of fertility: (1) normally fertile; (2) moderately fertile; and (3) almost completely sterile. The plants were classified according to these groups for each X-ray dosage given, and the results are given in the table below:

TABLE 8. TYPES OF PLANTS OBTAINED BY THE USE OF X-RAYED POLLEN

X-ray dosage (in r units)	Total no of plants at end of vegetative period	Number of plants				
		Of hybrid type	Of aberrant type	Of maternal type		
				Normally fertile	Moder- ately fertile	Sterile or almost sterile
12,000	132	7	116	0	3	6
15,000	167	9	127	4	11	16
17,000	17	0	16	0	0	1
19,000	16	0	15	0	0	1
21,000	2	0	1	1	0	0
23,000	2	0	0	2	0	0
Totals	336	16	275	7	14	24

As may be seen from Table 8, the great majority of the plants were aberrant in type. Comparatively very few of them belonged to the hybrid type, and this type of plant no longer appeared after the X-ray dosage reached 17,000 r . Maternal-type plants, on the other hand, constituted a fairly large group. They occurred with the greatest frequency at an X-ray dosage of 15,000 r . Out of a total of 125 plants grown from normal-appearing and shriveled seeds 24 were maternal in type; of 42 plants from very shriveled seeds 7 were of this type.

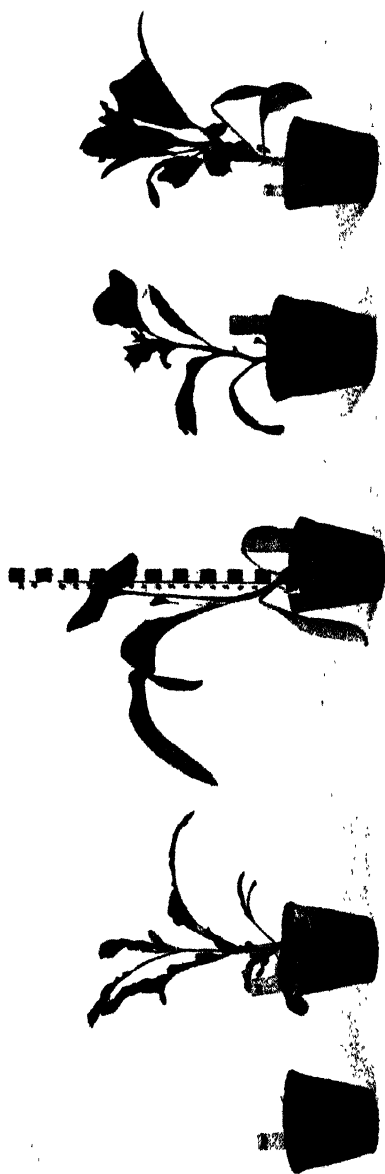
The group of aberrant-type plants was quite a miscellaneous group. It included all plants clearly abnormal in structure. These abnormalities were reflected, for the most part, in the character of branching and the shape of the leaves; also the growing together of leaf blades and stems in twos and threes was of quite frequent occurrence. As an example of an extreme case of abnormal leaves, we may take Plant No. 177 (Phot. 8), the width of whose leaves did not exceed 1.6 cm. For comparison, let us take Plant No. 4 (Phot. 5), a normal F_1 plant from the combination *N. rustica* var. *humilis* ♀ × *N. rustica* var. *texana* ♂. Moreover, the changes in leaf shape involved, to various degrees, loss of structural symmetry and frequently very marked curling of the leaves. Especially great asymmetry was shown



PHOT 5 Plant No. 4. — F_1 hybrid *rustica humilis* \times *N. rustica texana*. Plant No 251 — *N. rustica humilis* \times $1/4$ haploid. Plants Nos. 43 and 82. Aberrant type plants



PHOT. 6



PHOT. 7



PHOT. 6, 7, and 8 Aberrant-type plants grown from seeds obtained by the use of X-rayed pollen. $\times \frac{1}{4}$



PHOT. 9. Leaves grown together from aberrant-type plants. $\times c. \frac{1}{8}$.

in the case of Plants Nos. 27 and 31 (Phot. 6). Very often there were observed most fantastic contours of the leaves (Phot. 7, Plants Nos. 55, 191 and 76).

The growing together of leaves in twos and threes is shown in Photograph 9 (herbarium specimens), Photograph 7 (Plant No. 162), and Photograph 6 (Plants Nos. 154 and 41). Very often not only separate leaves but the entire upper portion of the plant was grown together, as shown in Photograph 6 (Plants No. 50 and 154). Most of the plants with such tops were unable to continue their growth, and ceased growing altogether during the remainder of the vegetative period; some continued growing but only with their side branches (Phot. 6, Plant No. 154).

Of interest also are the few extremely dwarf plants (Phot. 7, Plant No. 527, and Phot. 8, Plant No. 488). These dwarf plants had leaves somewhat aciculate in shape and scarcely any stems at all. By the end of the vegetative period their height was only 3–5 cm., and they had not blossomed.

The group of aberrant plants were characterized not only by the abnormal shape of their leaves but also by the abnormal development of their stems and by the growing of several stems together. Abnormal flower structure was most frequently expressed in cleft corollas or in the growing of two or three corollas together.

As regards fertility, aberrant plants were characterized, as was to be expected, by a high degree of sterility, the number of completely sterile plants increasing with the increase in the X-ray dosage given the pollen. In Table 9 all the aberrant plants are subdivided, for each respective X-ray dosage, into plants: (1) slightly sterile, (2) moderately sterile, (3) highly sterile, and (4) completely sterile. The degree of sterility was determined by the unaided eye, on the basis of the number of capsules and seeds, at the close of the vegetative period.

As may be seen from this table, only a single plant was classified as "slightly sterile". Most of them (181 out of 275) were completely sterile, 53 were highly sterile, and 40 moderately sterile.

The large percentage of abortive pollen (Table 10) — revealed, in plants selected at random, by analyses made by the aceto-carminic method — shows that the high sterility may be attributed to irregularities at meiosis.

TABLE 9. DEGREE OF STERILITY OF ABERRANT PLANTS

X-ray dosage (in units)	Total no of aberrant plants	Number of aberrant plants				
		Normally fertile	Slightly sterile	Moderately sterile	Highly sterile	Completely sterile
12,000	116	—	1	23	24	68
15,000	127	—	—	15	23	89
17,000	16	—	—	—	1	15
19,000	15	—	—	2	5	8
21,000	1	—	—	—	—	1
23,000	0	—	—	—	—	—
Totals	275	—	1	40	53	181

TABLE 10. DEGREE OF FERTILITY OF POLLEN OF ABERRANT PLANTS

Plant No	Total no of pollen grains	No of fertile pollen grains	No of abortive pollen grains	Fertile pollen (in %)	Plant No	Total no of pollen grains	No of fertile pollen grains	No of abortive pollen grains	Fertile pollen (in %)
12	1255	470	785	37.45	222	1243	90	1153	7.24
21	1219	504	715	41.35	236	1103	189	914	17.14
34	1274	413	861	18.90	237	1172	122	1050	10.41
80	395	131	264	33.16	238	1163	64	1099	5.50
86	1928	52	1876	2.70	239	1202	353	849	29.37
95	1396	86	1310	6.16	241	1157	69	1088	5.96
103	1263	622	641	49.25	244	1243	44	1199	3.54
107	1203	125	1078	10.39	249	1276	44	1232	3.45
111	684	30	654	4.39	252	1330	110	1220	8.27
112	1118	71	1047	6.35	258	1332	319	1013	23.95
113	1272	153	1119	12.03	260	1055	49	1006	4.64
114	1239	220	1019	17.76	271	1079	50	1029	4.63
121	1138	88	1050	7.73	273	1789	1069	720	59.75
122	1214	264	950	21.75	281	1172	94	1078	8.02
123	1470	404	1066	27.48	283	2225	1005	1220	45.17
125	1239	166	1073	13.40	480	1049	731	318	69.68
129	1172	607	565	51.79	482	1223	243	980	19.87

Plants of the hybrid type were readily distinguishable by their morphological characters. They were not deformed in any way, and were characterized by normal growth. They had broad leaves, in this respect showing almost complete dominance of the paternal form, the variety *texana*. Fertility of such plants was either normal or slightly below normal, indicating that in some cases these plants were also the result of chromosomal aberrations but apparently not of such an extreme nature as to affect their somatic structure.

The hybrid-type plants were of even less interest for our immediate task, viz., the production (and finding) of haploid plants, than those aberrant in type, since it was evident that their formation had been brought about by the participation of the paternal plant and, consequently, by fertilization.

The most interesting of the three groups of plants was, undoubtedly the group of maternal-type plants, for it is precisely among such plants that one should look for those which may have arisen parthenogenetically.

Maternal-type plants, in our case, were encountered comparatively often, but they were not all alike. As already mentioned, it was necessary, first of all to divide this group of plants into three, according to degree of fertility: (1) normally fertile, (2) moderately fertile, and (3) completely sterile.

In seeking for parthenogenetic plants the most promising were the normally fertile plants, since the presence of diploid parthenogenesis might be assumed, and those completely sterile, since these might arise as the result of generative parthenogenesis. On the other hand, the intermediate group, i.e., plants with moderate fertility, proved not so interesting, since their chromosome number was over 24. Neither could they be normal diploids, since sex-cell formation was irregular.

All plants in all three groups of "maternals" were carefully examined, and from each root-tips were taken and fixed for the purpose of chromosome counts. Because of the immensity of the task of making chromosome counts for such a large number of plants, we made careful counts only for a few plants, limiting ourselves as regards the others to the notation "over 24 chromosomes".

All plants with normal fertility had 48 chromosomes. Plants with reduced fertility had over 24 chromosomes, but whether or not they



PHOT. 10. a. *N. rustica humilis*, diploid; b. *N. rustica texana* diploid; c. maternal-type plant, obtained by pollinating flowers of the variety *humilis* with X-rayed pollen of the variety *texana*.



PHOT. 11. Leaves of diploid and haploid *N. rustica* var. *texana*.

$\times \frac{1}{8}$

all had exactly 48 was not determined. In one case, where it was easy to count the number of chromosomes, it was found to be 46, *i.e.*, there appeared to be a loss of two chromosomes. Examination of chromosome plates often revealed the presence of fragments.

Maternal-type plants characterized by complete sterility were also examined, and among them there was found one plant having 24 chromosomes, *i.e.*, a plant with a haploid chromosome set. All the rest had more than 24 chromosomes, and so were not haploids, although morphologically they resembled them. Thus, only one plant out of the 45 maternals proved to be a haploid (from the combination *humilis* ♀ × *texana* ♂).

The maternal-type plants with normal fertility are also of exceptional interest. Their origin, may be explained in one of two ways: they arose as the result either of apomixis or of "self-contamination" at the time of emasculation. Taking into account the frequency of occurrence of these plants, we are convinced that they arose as the result of apomixis.

In all we had seven normal diploid plants purely maternal in type. They arose from seed from different capsules. Thus, three plants were obtained from seeds of capsule No. 4286/4, two from normal-appearing and shriveled seed and one from very shriveled seed. The fourth maternal plant arose from very shriveled seed of capsule No. 4288/1 (Phot. 10, Plant No. 521). All these four plants represent progeny from pollination with pollen given an X-ray dosage of 15,000 *r*. At a dosage of 12,000 *r* no such plants were encountered; nor were any found, when the dosage was 17,000 or 19,000 *r*. With still heavier doses we obtained only two capsules with seeds: with a dosage of 21,000 *r* capsule No. 4300/2 and with a dosage of 23,000 *r* capsule No. 4308/2. From the former two plants were grown, one of them an aberrant plant and the other purely maternal as regards all its characters and normally fertile. Capsule No. 4308/2 had only two seeds, from which two normal maternal plants were produced.

There is no doubt but that all the seven plants maternal with respect to morphological characters and possessing normal fertility arose as the result of apomixis. Otherwise it would be difficult to account for their occurring singly (and among progeny obtained by the use of pollen given heavy X-ray doses), and for the fact that of the total seven plants three arose from very shriveled seeds, *i.e.*, seeds with scarcely any endosperm.

It remains unclear to us only which precise kind of apomixis took place, since the diploid maternal plants might have arisen as a result of somatic (diploid) parthenogenesis, apogamy, or, possibly, as the result of generative (haploid) parthenogenesis with subsequent doubling of the chromosome number during the first stages of embryonic development. The latter is the more probable, since we noted that the *N. rustica* haploids had diploid cells in their tissues, indicating the presence of some process capable at times of causing the chromosome set to revert to its diploid state.

Investigations as to set of seed, germinating capacity of seeds, ability to survive of seedlings, and morphology of mature plants were carried out in detail only for the combination *N. rustica* var. *humilis* ♀ × *N. rustica* var. *texana* ♂.

The reciprocal combination, i.e., *N. rustica* var. *texana* ♀ × *N. rustica* var. *humilis* ♂, was not investigated in such detail. As regards the relation between set of seed and X-ray dosage, it was found to be similar to that already described in the case of the first combination. The germinating capacity of the seeds was also very low and decreased with increased X-ray dosage. With respect to the morphological features of the plants obtained from this combination, when the pollen of the variety *humilis* was X-rayed, it should be noted that — while in the combination *humilis* ♀ × *texana* ♂ most of the plants were greatly deformed, particularly as regards leaf shape — in this case deformed plants were encountered much more rarely. Most of the plants were normal in morphological structure, and resembled either the mother plants (var. *texana*) or hybrids between the two varieties. However, they varied greatly in fertility, revealing thereby their aberrant condition.

Among the plants from this combination, where flowers of the variety *texana* were pollinated with X-rayed pollen of the variety *humilis*, three haploid plants were found. They were first detected by their purely maternal characters and their complete sterility. A subsequent chromosome count confirmed their haploid nature.

DESCRIPTION OF HAPLOID *Nicotiana rustica* PLANTS

Haploid plants of *Nicotiana rustica* — in two of its varieties, viz., var. *humilis* and var. *texana* — were obtained as the result of genera-

tive parthenogenesis artificially induced by pollinating emasculated flowers with X-rayed pollen (WINKLER's method).

The X-ray doses given the pollen, from the use of which haploid plants were obtained, were as follows: 17,000 *r* — in the case of the use of X-rayed *texana* pollen for the pollination of *humilis* flowers; 26,000 and 30,000 *r* — in the case of the use of X-rayed *humilis* pollen for the pollination of *texana* flowers.

Three haploids of the variety *texana* were found among 21 plants obtained by the use of X-ray doses of 26,000 and 30,000 *r*; and one haploid of the variety *humilis* among 17 plants obtained by the use of an X-ray dosage of 17,000 *r*. Thus, in the summer of 1935 we had at our disposal a total of four haploid plants, which were subjected to detailed analysis, both morphological and cytological.

Determination of the somatic chromosome number and study of meiosis in these plants were made by the aid of permanent preparations fixed with NAVASHIN's (10 : 4 : 1) and BOVIN-ALLEN's solutions and stained with HEIDENHAIN's iron-haematoxylin. Meiosis in pollen mother-cells was also studied by the aceto-carmin method, which, it may be noted, gave excellent results for observations beginning with the first metaphase, but the prophases, even in diakinesis, were not clear. Especially good results were obtained with aceto-carmin at the second metaphase, when it was comparatively easy to count the chromosomes grouped in regular plates at the poles.

For the cytological observations a Soviet microscope was used with a magnification of from 1,300 to 1,800. The drawings were made with the aid of an Abbé camera lucida.

Morphological description of N. rustica haploids

As regards their external characters, the *N. rustica* haploids, both of the variety *texana* and of the variety *humilis*, were very similar to the initial mother plants. In this respect the *texana* haploids revealed greater similarity, the *humilis* haploid differing somewhat as regards leaf shape. Its leaves were noticeably more elongated (Phot. 5, Plant No. 251). The leaves of the *texana* haploids were of exactly the same shape as those of the mother plants but very much reduced in size (Phot. 11).

The haploids were less vigorous than diploids. The *humilis* haploid



Рис. 12 *N. rustica* var. *texana* (diploid ($2n = 48$)). $\times c. 1/8$.



PHOT. 13. *N. rustica* var *texana* haploid ($2n = 24$).
 $\times c. \frac{1}{8}$.

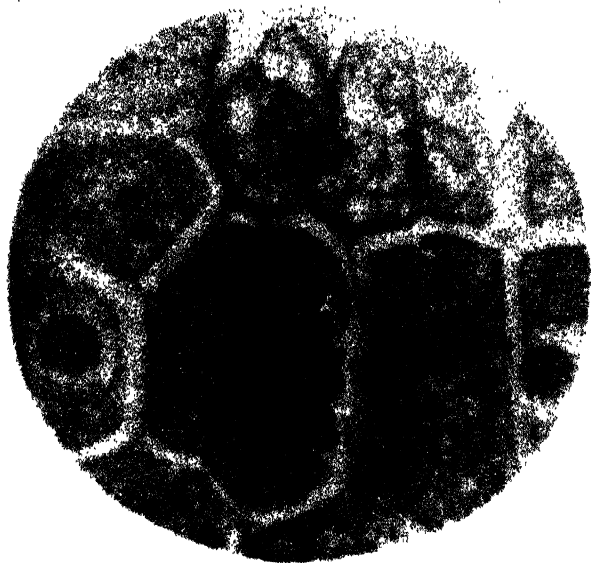
(Plant No. 251) was grown all the time in a pot without being transplanted to the field. For comparison one normal plant was also left in a pot. The height of the haploid was 35 cm., while that of the diploid was 42 cm. The other haploids likewise differed but slightly in height from diploid plants. One of the *texana* haploids (Plant No. 645) was set out in the field at the same as the diploid plants. Its height (90 cm.) was not less than that of the diploids, but vigor and extent of branching were, nevertheless, much reduced. Whereas the diploid plants of the variety *texana* were vigorous, well-developed bushes, the haploid plant, grown under the same conditions and having the same height, had only one well-developed stalk and a single side branch (see Phot. 12 and 13).

In Table 11 (page 334) measurements are given of meristematic cells and their nuclei in roots of haploid and diploid *N. rustica* plants. These measurements show that halving the number of chromosomes leads to halving the size of the nuclei and to almost halving the size of the cells (reduced by one-third to almost one-half). The length of the guard cells of the lower leaf surface of the haploid plants was also considerably shorter than that of similar cells in diploid plants.

At the time of flowering all the haploid plants proved to be almost completely sterile. Analyses of pollen fertility in the haploids (Table 12, page 335) gave very similar results for all four plants. Thus, if in normal diploid plants mature anthers have 92-97 per cent of fertile pollen, in the haploids they had only 2.42-2.97 per cent. This very small percentage of fertility of the pollen of the haploid plants determined their sterility, which was, of course, very high, since the percentage of fertile egg-cells was, presumably, not greater, and, consequently, the chances of a fertile male generative cell uniting with a fertile egg-cell were very small, especially in the case of self-pollination.

Almost all the flowers, together with their stems, dropped off on the fifth or sixth day after the opening of the corolla. But, nevertheless, the *N. rustica* haploids were not completely sterile, since we succeeded in obtaining a few seeds by open pollination and also a few by pollinating one of the haploids with pollen from *N. paniculata* ($2n = 24$). By open pollination from one to seven capsules set, containing a small number of seeds (see Table 13, page 337).

Unfortunately, in the present paper we cannot give a full analysis



PHOT. 14. Somatic chromosomes of an *N. rustica* haploid.
Ocular $20\times$, objective $101\times$.



PHOT. 15. First metaphase plate at meiosis of egg mother-cell.
Ocular $20\times$; ob. $101\times$.

TABLE 11. COMPARATIVE MEASUREMENTS OF CELLS IN HAPLOID AND DIPLOID *N. rustica* PLANTS

Plant	Measurements of nuclei of meristematic cells				Measurements of meristematic cells				Measurements of guard cells			
	No. of observations	Average diameter (microns)	Ratio of diameters (n : 2n)	Average volume (cu. microns)	Ratio of volumes (n:2n)	No. of observations	Average area (sq. microns)	Average height (microns)	Average volume (cu. microns)	Ratio of volumes (n : 2n)	No. of observations	Average length (microns)
<i>N. rustica</i> v. <i>texana</i>												
Diploid	200	11.98 ± 0.059	—	899.87	—	100	1093.28 ± 27.800	11.84 ± 0.181	12944.44	—	519	46.168 ± 0.181
Haploid (No. 555) . .	500	9.14 ± 0.039	1.31	399.62	2.3	100	687.75 ± 14.670	10.76 ± 0.113	7400.19	1.17	—	—
" (No. 660) . .	500	8.91 ± 0.035	1.34	371.46	2.4	100	643.60 ± 11.120	10.98 ± 0.151	7066.73	1.18	523	26.319 ± 0.141
" (No. 645) . .	515	9.88 ± 0.040	1.21	504.76	1.8	100	752.40 ± 13.424	10.54 ± 0.130	7930.29	1.16	519	124.702 ± 0.116
<i>N. rustica</i> v. <i>humilis</i>												
Diploid	216	12.01 ± 0.069	—	904.39	—	100	971.33 ± 26.724	12.03 ± 0.178	11685.10	—	600	37.99 ± 0.165
Haploid (No. 251) . .	110	9.27 ± 0.084	1.30	416.86	2.2	100	756.32 ± 18.731	10.66 ± 0.199	8062.37	1.45	600	123.27 ± 0.136

TABLE 12. FERTILITY OF POLLEN OF THE HAPLOIDS

Plants	No of pollen grains		Fertile pollen (in %)
	Sterile	Fertile	
Diploids:			
<i>N. rustica</i> var. <i>humilis</i>	173	2092	92.4
<i>N. rustica</i> var. <i>texana</i>	39	1207	96.9
Haploids:			
<i>N. rustica</i> var. <i>humilis</i> (No. 251) .	2023	58	2.79
<i>N. rustica</i> var. <i>texana</i> (No. 555) .	2003	55	2.67
“ “ (No. 645) .	1976	49	2.42
“ “ (No. 660) .	1991	61	2.97

of the progeny of the haploids obtained by open pollination nor of the F_1 plants from the cross haploid *N. rustica* var. *humilis* ♀ × *N. paniculata* ♂, since this will be possible only later, after the close of the vegetative period and after a thorough cytological investigation. But it can already be stated that the F_1 plants (haploid *N. rustica* ♀ × *N. paniculata* ♂) are very clearly hybrid in character, and that at meiosis of the pollen mother-cells there are observed 12 bivalents and 12 univalents (Plate 1, Figs. 7 and 8).

The seeds from the cross haploid *N. rustica* ♀ × *N. Langsdorffii* ♂ proved to be entirely inviable. The progeny of the haploids obtained by open pollination was, for the most part, diploid and normally fertile; however, a few polyploid plants were found. Their chromosome numbers have not yet been precisely determined, except in the case of one plant. This plant proved to have 72 chromosomes in its somatic cells ($2n = 72$), i.e., to be a triploid *N. rustica*.

As may be seen from the data in Table 13, we did not succeed in obtaining seed from the haploids by self-pollination nor by pollination with pollen from another haploid. Neither did our attempts to pollinate flowers of diploid plants with pollen from haploid plants prove successful. Seeds were obtained only by open pollination and by pollination of haploid flowers with normal pollen from diploid plants.

Hybridization of *N. rustica* haploids with diploid plants of other

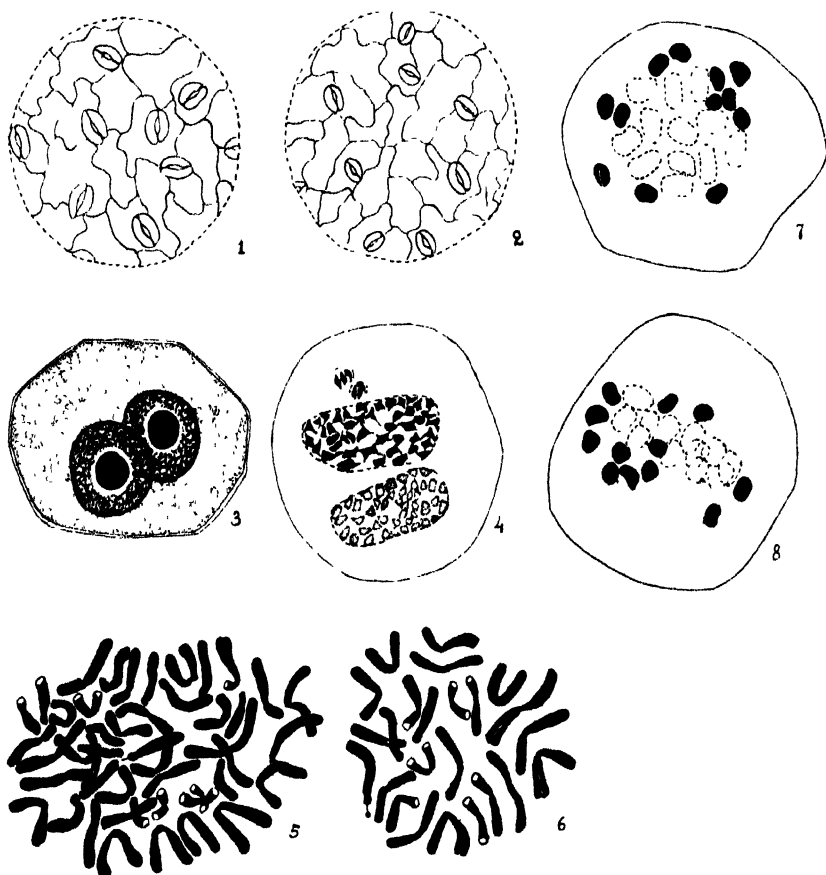


PLATE 1

FIG. 1 Epidermis of lower surface of a leaf of an *N. rustica* diploid

FIG. 2 Epidermis of lower surface of a leaf of an *N. rustica* haploid

FIG. 3 BINUCLEATE somatic cell of an *N. rustica* haploid

Ocular 20 \times , obj 101 \times

FIG. 4 Binucleate mother-cell of an *N. rustica* haploid at second telophase
45-3

Chromosome distribution | | Ocular 20 \times , obj 101 \times .
45 3

FIGS 5 and 6 Somatic chromosomes of diploid and haploid *N. rustica*.

Ocular 20 \times , obj 101 \times .

FIGS 7 and 8. First metaphase of pollen mother-cell of the hybrid, haploid
N. rustica ♀ \times *N. paniculata* ♂ (12n + 12n) Ocular 20 \times ; obj 101 \times .

TABLE 13. NUMBER OF SEEDS OBTAINED FROM *N. rustica* HAPLOIDS BY CROSSING AND BY OPEN POLLINATION

Mother plant ♀	Pollen parent ♂	No of flowers pollinated	Capsules obtained	Seeds obtained
Haploid var <i>humilis</i> (No 251)	Open pollination		3	24
Haploid var <i>texana</i> (No 555)	"	—	5	2
" " " (No 645)	"	—	7	67
" " " (No 660)	"	—	1	3
Haploid var <i>texana</i> (No 645)	Diploid var <i>humilis</i>	5	4	3
" " " (No 660)	"	3	2	8
Diploid var <i>humilis</i> . . .	Haploid var <i>texana</i>	25	0	0
" " " . . .	Haploid var <i>humilis</i>	25	0	0
Haploid var <i>texana</i> .	Self-pollination	10	0	0
Haploid var <i>humilis</i> .	Haploid var <i>texana</i>	10	0	0
Haploid <i>N. rustica</i> . . .	<i>N. Langsdorffii</i> (2n = 18)	4	2	5
" " . . .	<i>N. paniculata</i> (2n = 24)	11	6	95
" " . . .	<i>N. sylvestris</i> (2n = 24)	9	4	0
" " . . .	<i>N. glauca</i> (2n = 24)	7	3	0
" " . . .	<i>N. glutinosa</i> (2n = 24)	7	1	0
" " . . .	<i>N. suaveolens</i> (2n = 24)	8	0	0
" " . . .	<i>N. acuminata</i> (2n = 24)	10	0	0
" " . . .	<i>N. tabacum</i> (2n = 48)	6	4	0

species was successful only in one case, viz., in the combination haploid *N. rustica* ♀ × *N. paniculata* ♂. It is of interest to note that precisely in this case the very largest number of seeds were obtained, more than by open pollination of the haploids and more than by pollination with normal pollen from *N. rustica* diploids.

Number of chromosomes in the somatic cells of the haploids

The somatic chromosome number of all four haploids was 24 (Phot. 14; Plate 1, Figs. 5 and 6). Counts were made from a large number of good plates. A particularly detailed analysis was made of two haploid plants — one of the variety *texana* (Plant No. 660) and one of the

variety *humilis* (Plant No. 251). Special search was made for diploid cells in the haploid tissues, as previously described in the case of a number of haploids of other species. The results of this search are given in Table 14 below. For these counts permanent preparations were made of dozens of root tips from each of the haploids. Counts were made only of those cells whose chromosomes were at metaphase.

TABLE 14. FREQUENCY OF DIPLOID CELLS IN ROOT-TIPS OF *N. rustica* HAPLOIDS

Variety	No. of equatorial plates		Percentage of plates with a diploid no. of chromosomes (2n = 48)
	With a haploid no. of chromosomes (2n = 24)	With a diploid no. of chromosomes (2n = 48)	
Haploid var. <i>humilis</i>	496	13	2.555
Haploid var. <i>texana</i>	252	12	2.235

During this inspection several binucleate cells were found (Plate 1, Fig. 3), which, presumably, are the cause of the formation of diploid nuclei in haploid *N. rustica*, as reported by WEBBER (1933) for haploid *N. glutinosa* in his paper devoted in great part to this interesting problem. We did not succeed in finding such cells at the stage of spindle formation, but once both nuclei in a binucleate cell were found at the stage of late prophase, and they lay so close to each other that it would seem very probable to expect subsequent formation of a common equatorial plate and a common spindle.

The second possible cause of the formation of diploid nuclei in a haploid, described by WEBBER in the case of haploid *N. glutinosa* — doubling as a result of failure of the chromosomes to separate at anaphase and the inclusion of the entire diploid chromosome group in a single nucleus — we did not observe in our case. Mitotic division always proceeded normally. It should only be noted that the equatorial plates at metaphase in the haploids were not as regular as in diploids, the chromosomes being somewhat more scattered. To find so-called "good" plates was more difficult in the case of the haploids.

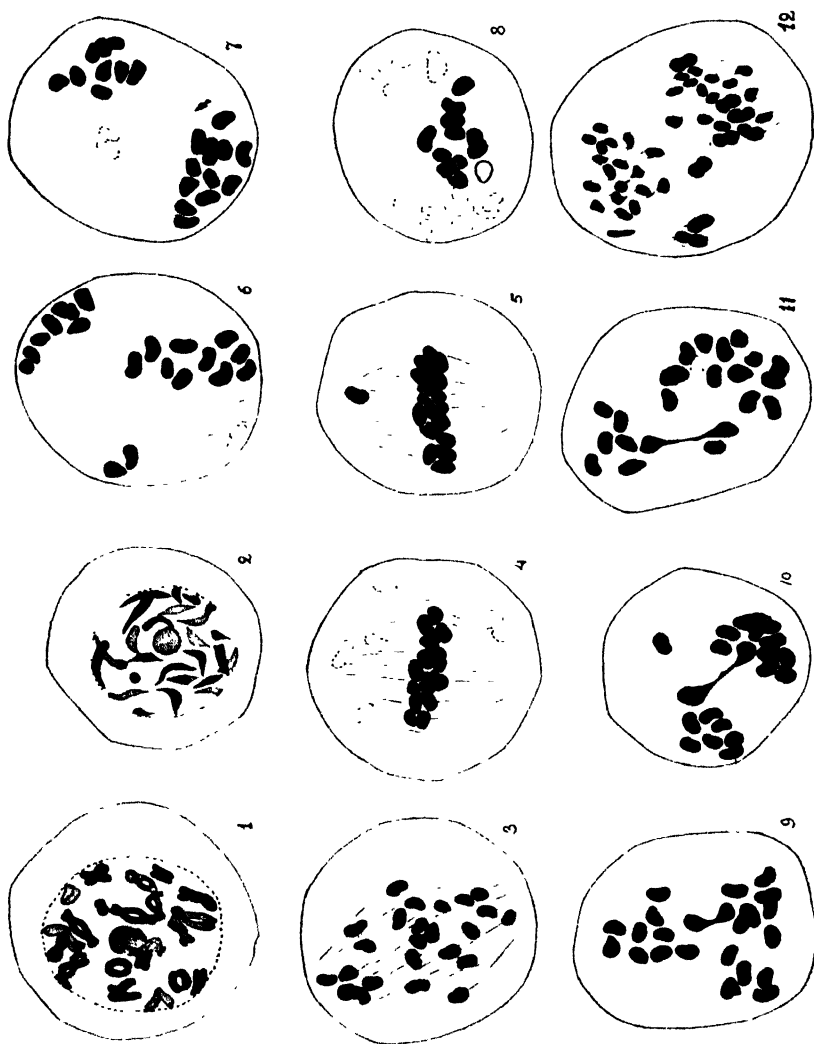


PLATE 2

FIG. 1. Diakinesis in a pollen mother-cell of an *N. rustica* diploid.

FIGS 2-12. Meiosis of pollen mother-cells of *N. rustica* haploids

2. Diakinesis. — 3-5. First metaphase — 6-8. Examples of chromosome distributions after the first division, showing several chromosomes lying outside the heterotypic spindle. — 9-11. Rarely occurring „bivalents” at first anaphase of the heterotypic division. — 12. Fusion of two spindles of the homeotypic division. On the common spindle are seen 6 undivided univalents and 32 half-chromosomes. A miniature spindle has been formed near two chromosomes which during the first division remained outside the spindle area.

Microsporogenesis in N. rustica haploids

Prophase in pollen mother-cells (PMC's) of *Nicotiana rustica* haploids proceeds, on the whole, normally, except for the absence of chromosome pairing. At early prophase separate threads were seen. To trace all the threads of the spireme was not possible, due to their large number, considerable length, and tangled state.

At the stage of late prophase (diakinesis) there could be very clearly seen fairly thick, bent single chromosomes, which, scattered about the periphery of the nucleus, were quite easy to count. There were always 24 of them. At diakinesis of diploid *N. rustica*, on the other hand, there are always 24 pairs of chromosomes, forming various configurations (Plate 2, Figs. 1 and 2).

Beginning with metaphase, meiosis in the haploids changes markedly, becoming very irregular. Of all the phases of meiosis metaphase of the heterotypic division is particularly irregular. At this phase the behavior of the chromosomes, which are in a univalent state, is most abnormal. The 24 univalent chromosomes are scattered over the entire spindle (Plate 2, Fig. 3). In the equatorial section of the PMC's there may, nevertheless, often be noticed a tendency of some of the univalent chromosomes to form a plate. Usually this group includes but a few of the univalents; only rarely are plates found including all or nearly all the univalents (Plate 2, Figs. 4 and 5).

A very large number, as many as one-third, of the PMC's have several chromosomes in the plasma outside the spindle area. The number of such univalent chromosomes not included in the heterotypic division ranges from 0 to 9. This irregularity in the heterotypic division complicates still more the formation of viable gametes, which in any case can only be of rare occurrence, due to the random distribution of the univalent chromosomes at first anaphase.

As is evidenced by the frequency of occurrence of PMC's with several chromosomes (from 1 to 9) lying in the plasma outside the spindle area at the heterotypic division (see Table 15), the laggards are not specific chromosomes, which particular ones remain outside the spindle being purely accidental.

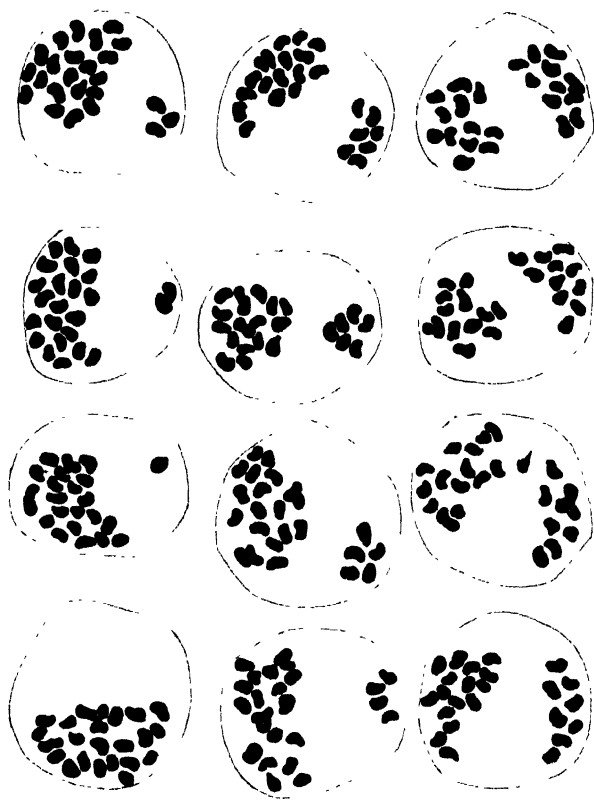


PLATE 3

Types of distribution of univalents as a result of the heterotypic division of pollen mother cells in *N. rustica* haploids (the distribution type „10-14” is not shown in the plate)

TABLE 15. NUMBER OF POLLEN MOTHER-CELLS WITH CHROMOSOMES LYING IN THE PLASMA OUTSIDE THE SPINDLE AREA AT FIRST METAPHASE

	Total number examined	Having all chromo- somes within the spindle area	Having chromosomes in plasma outside the spindle									
			Total	1	2	3	4	5	6	7	8	9
PMC's	327	218	109	34	21	19	13	8	5	5	3	1
In %	100.0	66.7	33.3	10.4	6.4	5.8	4.0	2.4	1.5	1.5	1.0	0.3

At first anaphase the univalent chromosomes within the spindle area are distributed at random to the poles. This random distribution of the chromosomes leads to the formation of unequal groups at the poles. The univalents lying outside the spindle remain, apparently, motionless, or are included in one of the groups, if it is formed in close proximity. Not once did we observe at first anaphase the division of a univalent, but, nevertheless, this phenomenon evidently occurs, though only very rarely. Thus, later, when making chromosome counts at second metaphase, out of 620 PMC's examined two had the following chromosome distribution, 10-1-14 and 11-2-12, which could occur only if one of the univalent chromosomes had divided into two halves at first anaphase, since these PMC's had a total of 25 chromosomes.

Consequently, if we disregard the rarely occurring instances of divided chromosomes, it may be assumed that, as a rule, the chromosomes at the heterotypic division are distributed as wholes. Chromosome counts at second metaphase confirmed this assumption, and also confirmed the random distribution to the poles observed at first anaphase (Table 16). For at second metaphase we found all possible variations in the distribution of the 24 elements to the two poles — from 0-24 to 2-12 (Plate 3, Figs. 1-12). The chromosome counts of second metaphase plates, given in Table 16, were made only for those cells in which all the chromosomes were included in the heterotypic spindle and none were left in the plasma outside the spindle area.

TABLE 16. DISTRIBUTION OF UNIVALENT CHROMOSOMES AT SECOND MEIAPHASE

Type of distribution of chromosomes	No of pollen mother-cells	Type of distribution of chromosomes	No of pollen mother-cells
0-24	24	7-17	34
1-23	28	8-16	44
2-22	28	9-15	82
3-21	34	10-14	82
4-20	22	11-13	92
5-19	26	12-12	98
6-18	26	Total	620

The PMC's with a 0-24 distribution may later produce two viable microspores, and, if it is assumed that each of the PMC's with other types of chromosome distribution will produce four microspores, then, based on the data of Table 16, we may calculate that out of the 620 PMC's there should be formed 48 microspores with 24 chromosomes and 2,384 with a fewer (*i.e.*, incomplete) number of chromosomes. Viable microspores (with 24 chromosomes) would in this case constitute 2 per cent of the total number, which closely approximates the percentage of fertile pollen (2.4-2.9%), determined by the acetocarmine method (Table 12), in mature anthers of haploid plants.

Those PMC's which at the heterotypic division have chromosomes not included in the spindle will form at second metaphase not two but several plates.

After the second metaphase meiosis proceeds more regularly, *i.e.*, at second anaphase the chromosomes, having split, are distributed to the poles of their respective spindles. Occasional chromosomes scattered in the plasma also often form miniature spindles, but sometimes they simply split into two halves, both of which are included in one small nucleus.

As a result of such chromosome behavior at meiosis, a large number of PMC's have at second telophase many nuclei (more than four), and these nuclei are very diverse in size, from very small to normal. The size of the nuclei is determined by the number of chromosomes contained in each.

At second anaphase we noted several cases of tri-polar spindles. Usually a major spindle of the PMC was joined by one pole to a miniature spindle of chromosomes lying in the plasma. Once (Plate 2, Fig. 12) we found a cell having one common spindle at second anaphase.

Among the irregularities at second anaphase should also be mentioned lack of synchronism in the separation of the chromosomes in a cell. Thus, while in one part of a PMC the chromosomes may have already split into halves and be separating to opposite poles, in another part of the same cell other chromosomes may only be commencing preparations for separation. Frequently there are lag-gard chromosomes lying scattered in the plasma. This lack of synchronism in the separation of the chromosomes at second anaphase causes the time of the beginning of telophase to be irregular.

The presence of binucleate cells in the somatic tissue of the haploids made it seem probable that binucleate PMC's might also be encountered. Therefore, in studying microsporogenesis, we tried to find such cells. We succeeded in finding only one. This PMC was already at the stage of telophase, but the chromosomes in each nucleus were still clearly visible. There were two nuclei with 45 chromosomes and two with 3 chromosomes (Plate 1, Fig. 4). The formation of such nuclei we explain in the following manner: A binucleate PMC, having in each of its nuclei 24 chromosomes, apparently formed during the first division a common spindle with 48 univalents; at first anaphase these univalents passed to the poles with a distribution of 3-45; subsequent

3 45

normal homeotypic division gave: $\begin{array}{cc} | & | \\ 3 & 45 \end{array}$.

All the irregularities at meiosis of the PMC's of haploid plants result, in the final analysis, in the formation of a great majority of inviable microspores. An examination of spores immediately after meiosis revealed their great diversity in shape, size, and number of nuclei (Plate 4, Figs. 1-12). Very many spores contained several nuclei, sometimes very small. In a number of spores we found from 1 to 9 micronuclei. Sporads (WEBBER, 1933) consisted of a varying number of spores, as may be seen from Table 17:

TABLE 17. FREQUENCY OF OCCURRENCE OF SPORADS OF VARIOUS TYPES IN *N. rustica* HAPLOIDS

Type of sporad	Number	Per cent
Monads	8	4.9
Diads	48	29.6
Triads	32	19.8
Tetrads	62	38.3
Pentads	6	3.7
Hexads	4	2.5
Above hexads	2	1.2
Total	162	100.0

The greatest number of sporads were tetrads. These, presumably, were the result of the division of cells in which all the chromosomes at the heterotypic division were included in the spindle, but sometimes even among them — though, of course, more rarely — there were found spores with several small, supplementary nuclei.

The diads, constituting 29.6 per cent of the sporads, may have arisen as the result of a 0-24 chromosome distribution, but not only in this way. In the first place, the frequency of their occurrence is greater than that of the 0-24 type of chromosome distribution, and, in the second place, very many of them had several nuclei each. Apparently, in those PMC's, in which at telophase two large and several small nuclei are formed, there may develop only one cell wall separating the two large nuclei from each other, the small nuclei being included in one or the other of the diad cells, depending upon which of the large nuclei they are nearest.

In a similar way we explain the formation of triads, which in our haploids constituted 19.8 per cent of the total. Sporads containing more than four spores (pentads, hexads, etc.) originated from cells having chromosomes lying outside the spindle in groups, not scattered throughout the plasma. As many as eight spores were observed in one sporad.

It is interesting that in the *N. rustica* haploids there occurred a few monads — only a small percentage (4.9%), it is true — the origin of which may be explained either by meiosis having been cut short

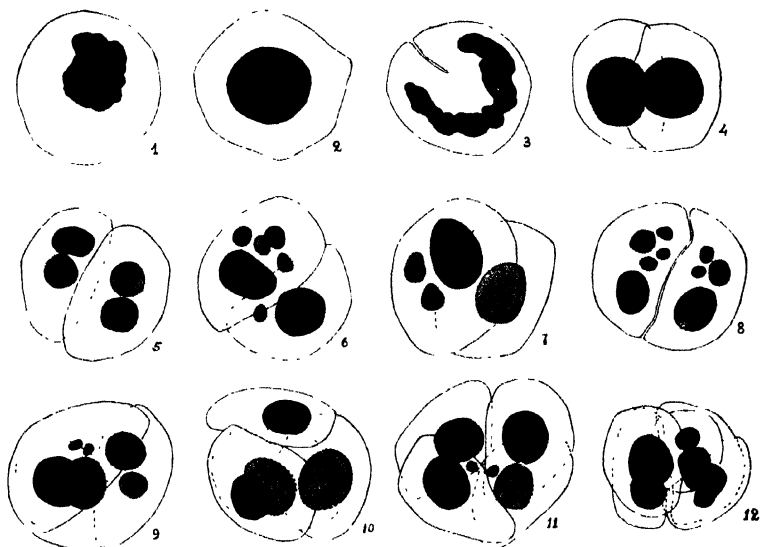


PLATE 4. TYPES OF SPORADS OF HAPLOIDS

FIGS 1-3 Monads. FIGS. 4-8 Diads. FIG. 9. Triads FIGS. 10-11 Tetrads FIG 12 Pentads Ocular $15\times$, obj $101\times$

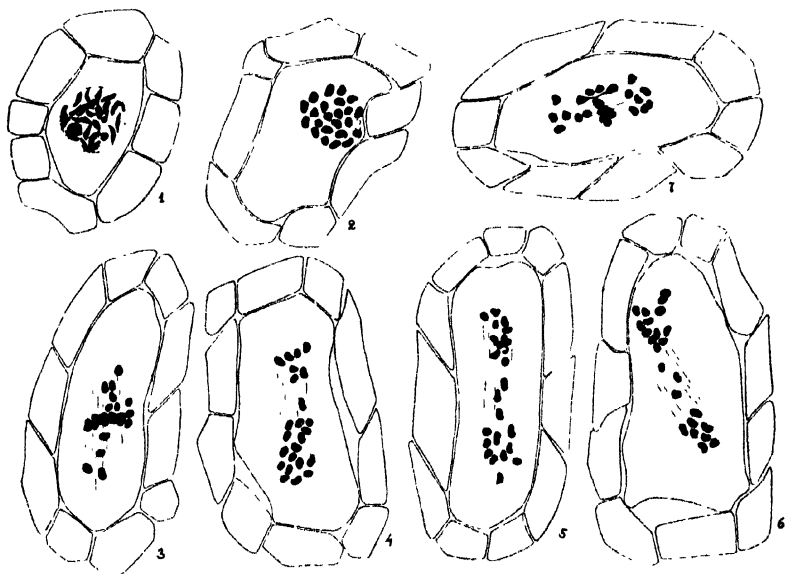


PLATE 5. Meiosis of egg mother-cells.

FIG. 1. Diakinesis FIG. 2. Metaphase of the heterotypic division. FIGS. 3-6. Anaphase of the heterotypic division. FIG. 7. First anaphase with one „bivalent”.

(Plate 4, Figs. 1 and 2) or by the failure of divided chromosomes to separate and the inclusion of the entire group in one nucleus (Plate 4, Fig. 3)

As already mentioned in describing meiosis and as shown in the figures, the nuclei in the sporads are very diverse as regards the number of chromosomes which they contain and, consequently, as regards their size. The table of measurements of spore nuclei in haploids and diploids is very significant in this connection:

TABLE 18. DIAMETERS OF NUCLEI IN SPORADS OF HAPLOID AND DIPLOID *N. rustica*

Plants	Frequency of occurrence of nuclei with diameters measuring (in microns)										Average diameter ($M \pm m$)
	1	2	3	4	5	6	7	8	9	10	
Haploids	11	16	28	44	67	36	6				4.758 ± 0.0073
Diploids							34	41	24	1	8.370 ± 0.0783

It is characteristic that the spore nuclei in diploids vary little in diameter — from 7 to 10 microns, while those in haploids vary from 1 to 7 microns. Of special interest are those spore nuclei in haploids which are of approximately the same size as those in diploids, *i.e.*, nuclei having a diameter of 7 microns. Here one may assume with most assurance the presence of all 24 chromosomes, as in the diploid. The percentage of such nuclei in the spores of haploids (about 3%) approximates the percentage of its fertile pollen, already cited, and also that of the 0/24 type of chromosome distribution at the heterotypic division.

The chief characteristic of microsporogenesis in *N. rustica* haploids, consequently, is the univalent state of the chromosomes at the heterotypic division. The absence of chromosome conjugation is the cause of the random distribution of the chromosomes to the poles and the subsequent formation of spores unequal in size and, for the most part, inviable. As a rule, at meiosis of *N. rustica* haploids chromosome conjugation is entirely absent, *i.e.*, all 24 chromosomes are univalents, but out of the great number of PMC's which we examined ten were found with obvious conjugation of one pair of chromosomes (Plate 2,

Figs. 9, 10, 11). These apparently rare cases may be regarded as exceptions, but, nevertheless, they are not without interest, since they indicate the possibility of the conjugation of non-homologous chromosomes in *N. rustica*.

Megasporogenesis in N. rustica haploids

Megasporogenesis in *N. rustica* haploids does not differ essentially in its irregularities from microsporogenesis. In the egg mother-cells (EMC's), just as in the pollen mother-cells (PMC's), meiosis proceeds with extreme irregularity. In the heterotypic division the 24 univalent chromosomes are distributed at random to the two poles. This random distribution of the chromosomes may be seen in Figures 4, 5 and 6 of Plate 5.

A peculiarity of meiosis in EMC's is the more regular course of first metaphase. Here, in most cases, all 24 univalent chromosomes are concentrated in the equatorial region of the cell and form a regular equatorial plate (Plate 5, Figs. 2 and 3). However, even here cases are not rare in which several chromosomes are scattered over the spindle or even lie in the plasma outside the spindle area.

The first anaphase of megasporogenesis proceeds in precisely the same fashion as that of microsporogenesis. Here also there were encountered a few mother-cells in which not all the chromosomes were univalents, one pair exhibiting obvious conjugation (Plate 5, Fig. 7). But this was a rare phenomenon. As a rule, chromosome conjugation was entirely absent. As a result of the irregular, random distribution of the chromosomes to the poles, the EMC's produce, for the most part, inviable megaspores. Usually all that was seen on the permanent preparations were already dead, degenerated megaspores (Plate 6, Figs. 1 and 2), only rarely were there encountered ovules in which was clearly visible the beginning of formation of an embryo sac in one of the megaspores. All the cases of the formation of embryo sacs which we observed in ovaries of *N. rustica* haploids occurred when only one or two megaspores were present. Apparently, here also, as in microsporogenesis, those spores prove viable which have received in their nuclei all 24 chromosomes, *i.e.*, those formed as the result of a 0 24 chromosome distribution or as the result of non-reduction of the chromosomes at the first division.

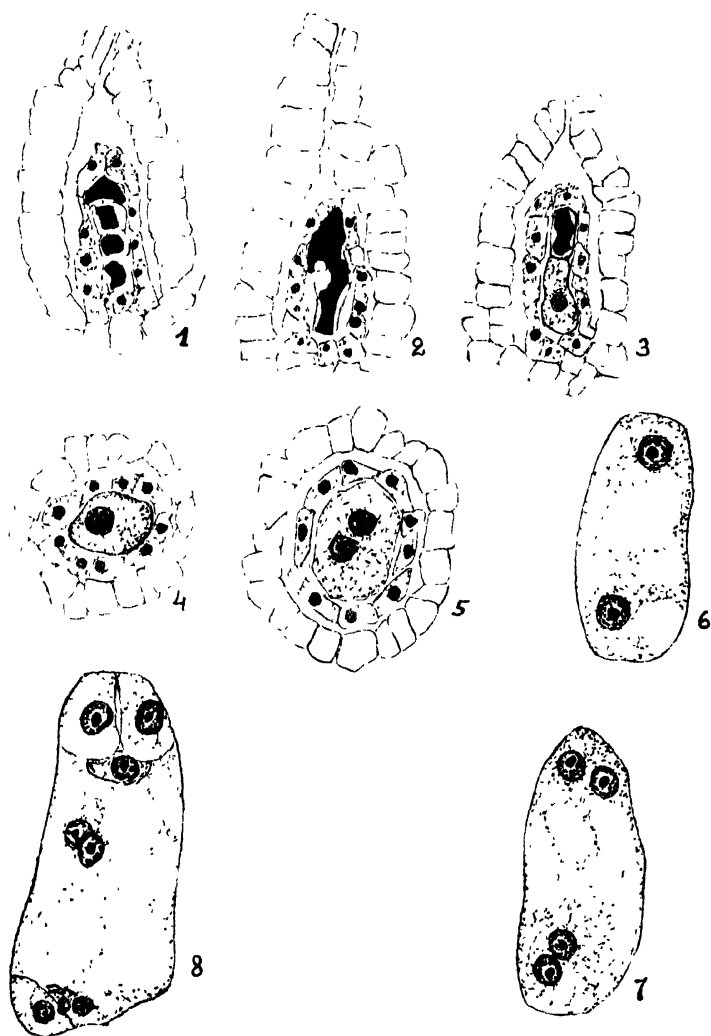


PLATE 6

Development of embryo sacs in haploid *N. rustica*

Figs. 1-3 Degeneration of megaspores.

Figs. 4-7 Embryo sacs with one, two, and four nuclei.

Fig. 8 A completed 8-nucleate embryo sac

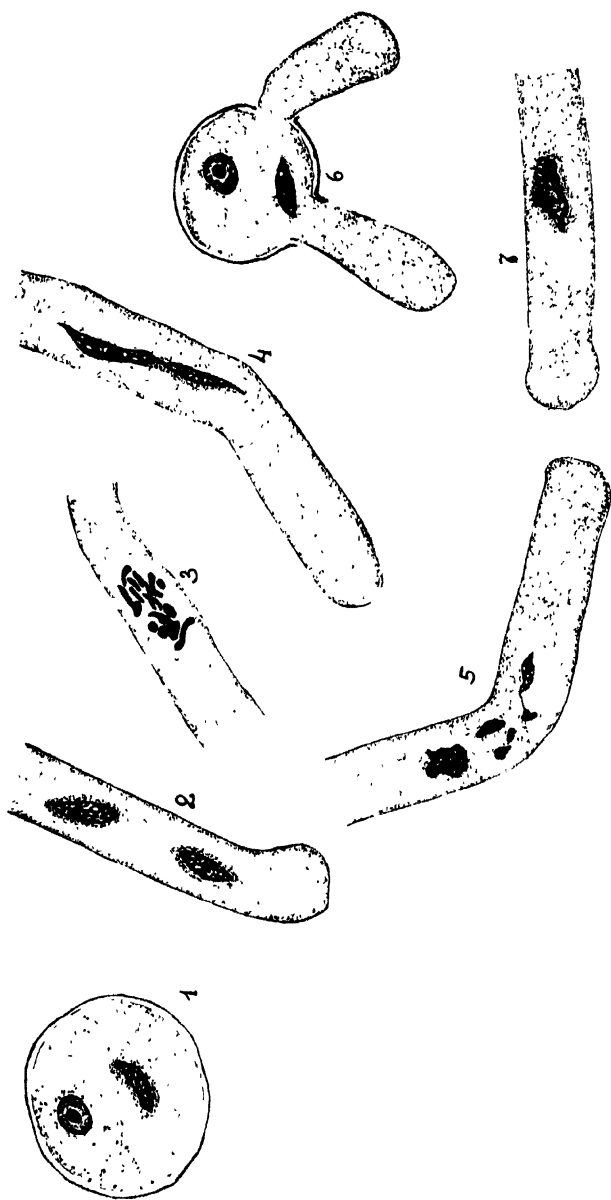


PLATE 7

Irregularities in the formation of generative nuclei in tubes of X-rayed pollen (dosage: 15,000-26,000r).
 FIG. 1. Normal pollen grain after first division of nucleus. There is visible one vegetative and one generative nucleus.
 FIG. 2. End of normal pollen tube with two generative cells
 FIGS. 3-7. Tubes of X-rayed pollen: 3. Second division of generative nucleus; chromosomes clearly deformed. —
 4. Non-division of generative nuclei. — 5. Formation of several micronuclei as a result of the second division. —
 6. Pollen grain with two tubes. — 7. Omission of second division.

The formation of embryo sacs (Plate 6, Figs. 4, 5, 6, 7 and 8) in the ovules of haploids, though of very rare occurrence, proceeds just as in normal diploid plants. One of the two megaspores of the haploid increases greatly in size, and, after the subsequent division of the nucleus, forms an octonucleate embryo sac. The great majority of the megaspores perish prior to the formation of embryo sacs, presumably soon after the heterotytic division.

It is interesting to note that the irregularities of megasporogenesis with respect to the formation of sporads — the formation of multi-nucleate spores with several micronuclei, the formation instead of diads (as at normal meiosis of EMC's) of monads, diads, triads, and larger groups of spores — repeat almost exactly the irregularities of microsporogenesis, only they occur less frequently. Considerably more often are megaspores encountered in the normal number, two. Possibly this is due to the more regular formation of an equatorial plate at first metaphase of meiosis in EMC's and, consequently, of the less frequent occurrence of mother-cells having chromosomes lying in the plasma outside the spindle area at the heterotytic division.

Unfortunately, our observations as to the frequency of occurrence of megaspores in monads, diads, triads, tetrads, etc. cannot be set forth in tabular form, as we have done in the case of microsporogenesis, because of the difficulty in obtaining the necessary material in sufficiently large quantities.

DISCUSSION

In taking up a discussion of our *N. rustica* haploids, it seems advisable to re-examine all the haploids obtained by various investigators, in order to make it possible to determine the similarities and differences between our haploids and haploids of other species.

For convenience in comparison we considered that it would be useful to attempt, on the basis of a careful perusal of a large number of original papers on experimental haploidy in flowering plants, to classify all haploids according to their chief distinguishing characteristics, the latter being, in our opinion, genomic constitution and meiotic irregularities.

As a result of such a classification of haploids, our *N. rustica* haploids will occupy a more definite place among the whole group

of haploids, and also all the literature reviewed by us will be presented in condensed form, easy of access for investigators working in this field.

Mode of origin of haploids in flowering plants

Fourteen years have passed by since the first haploid among flowering plants, *Datura stramonium* L., was discovered and described. During this period the number of haploids reported has grown apace, and now they are known among a considerable number of different species of widely separated families: *Solanaceae*, *Gramineae*, *Compositae*, *Malvaceae*, *Cruciferae*, *Portulacaceae*, and *Onagraceae*. From this it may be concluded that the possibilities of obtaining haploids in the higher plants are very extensive.

Haploids originate, for the most part, as the result of generative parthenogenesis or androgenesis (merogony). Among flowering plants neither kind of apomictic development is normal, and must be induced by some agency or other or by definite, specific conditions. And yet, no matter how far in the course of evolution the higher flowering plants have proceeded in the way of adaptation of their reproductive organs to the sexual process (amphimixis), spontaneous development of egg-cells without fertilization is, apparently, possible. In this connection the observations of KATAYAMA (1933), on the ninth day after emasculation, of developing embryos in unfertilized ovaries of *Triticum monococcum* are of interest. In one case he even succeeded in determining the chromosome number (7) of such an embryo, which thus proved to be haploid.

Spontaneous occurrence. — A comparatively large percentage of the haploids so far known arose spontaneously in pure lines, in field cultures, and among hybrid populations from intervarietal and interracial crosses. A list of such haploids follows:

1. In pure lines and in field cultures.

Oenothera franciscana (DAVIS and KULKARNI, 1930);

Nicotiana tabacum (KHRISTOV, 1930);

“ *glutinosa* (GOODSPEED and AVERY, 1929; WEBBER, 1933);

Oryza sativa (MORINAGA and FUKUSHIMA, 1931, 1932, 1934);

Brassica Napella (MORINAGA and FUKUSHIMA, 1933);

- Gossypium barbadense* and *G. hirsutum* (HARLAND, 1936),
Triticum monococcum (KIYARA and KATAYAMA, 1932, 1933, CHISAKI, 1933, KATAYAMA, 1935),
Triticum vulgare (YAMASAKI, 1934, 1936),
Hordeum vulgare (JOHANSEN, 1934).
 2. In hybrid populations from intraspecific crosses.
Lycopersicum esculentum (LINDSTRÖM, 1929, LINDSTRÖM and KOOS, 1931, HUMPHREY, 1934),
Portulaca grandiflora (OKURA, 1933),
Matthiola incana (LESLEY and FROST, 1928).

As regards spontaneous occurrence, of special interest are the cotton haploids, which, according to HARLAND (1936), regularly appear in every field of Sea Island cotton in a proportion of about one to every 3,000 or 4,000 normal plants. Spontaneous haploids in *Triticum monococcum* are also not of rare occurrence. According to KATAYAMA, they occur in field cultures to the extent of 0.48 per cent of the total number of plants.

More detailed investigations, of course, might disclose even here some cause or other inducing the parthenogenetic development of the egg-cells, but the examples given of such spontaneous occurrence of haploids in themselves indicate that the egg-cells of flowering plants have preserved the ability to develop without fertilization and do so develop under certain definite conditions.

Induction as a result of interspecific hybridization Crossing distantly related species greatly promotes the occurrence of haploids. The great majority of haploids among flowering plants have been obtained as a result of interspecific hybridization (see Summary Table, Appendix I). These haploids include the following species: *Datura stramonium*, *Oenothera franciscana*, *O. Hookeri*, *O. argillicola*, *O. blandina*, *O. rubricalyx*, *Solanum nigrum*, *Nicotiana tabacum*, *N. sylvestris*, *N. Langsdorffii*, *N. rustica* (KOSOFF, 1936), *Crepis capillaris*, *Triticum compactum*, *Tr. durum*, *Tr. turgidum*, *Gossypium Davidsonii*. Moreover, haploids of most of the foregoing species have been found repeatedly in different combinations, not merely in single instances.

The egg-cells of the mother plants are stimulated to development in this case, apparently, by the high degree of incompatibility between the male and female gametes, often making their fusion

impossible. According to the opinion of some of the investigators who have obtained haploid plants as a result of interspecific crosses, the egg-cell is stimulated to development by the pollen tubes of the other species. According to others, it is not the pollen tube as a whole which constitutes the stimulus but the sperm nucleus which has penetrated the embryo sac of the mother plant. JORGENSEN (1928) from a study of embryological material has described quite convincing cases of egg-cells stimulated by male gametes which have penetrated the embryo sac. He also observed cases of the sperm nucleus of one species penetrating the egg-cell of another species but not fusing with the egg-cell's nucleus and degenerating and being eliminated during the first divisions of the cell.

It seems to us that the question as to precisely what stimulates parthenogenetic development of the egg-cell in interspecific hybridization is far from being solved. Detailed embryological investigations are necessary in connection with the process of obtaining haploids. Our experiments on pollinating emasculated flowers with normal pollen, followed by the removal, after varying periods, of the styles containing the growing pollen tubes, are likewise not adequate to solve this very important and exceptionally difficult problem. But, in any case, we can definitely state that pollen tubes prior to penetration of the embryo sac do not have any stimulating effect on the development of egg-cells in *Nicotiana rustica*. In these experiments of ours there was not a single case of parthenogenetic development of embryos induced by growing pollen tubes. The location of the tubes with respect to the embryo sacs varied greatly, some of them having reached the very entrance to the embryo sac, but, nevertheless, there was no stimulation of the egg-cells. Moreover, some pollen tubes, having already penetrated the ovary, were broken in two when the style was removed, and their remnants in the ovary might have had for some time thereafter a fermentative effect.

All this forces us to regard with considerable skepticism the view that an effect may be produced on egg-cells directly by the pollen tubes without the active participation of the sperm nuclei.

In this light it can be more readily understood why the most effective crosses, as regards the production of haploids, are those from which it is very difficult to obtain hybrid progeny, those obtained often proving inviable and perishing in the seedling stage.

Induction by pollination with pollen from aberrant plants --- A few haploids have been obtained by pollinating normal plants with pollen from aberrant forms having a large percentage of abortive pollen or by crossing highly sterile mutants. A case of the latter has been described by NAKAMURA (1933) in rice. In *Nicotiana glutinosa* haploids were found in the F_3 from a cross between X-ray aberrants (WEBBER, 1933). In *Pharbitis Nil*, by pollinating the flowers of a normal plant with pollen from a periclinal chimaera (with a high percentage of abortive pollen grains), a haploid plant was also obtained (U, 1932).

It is quite evident that these cases, in our classification of haploids according to the cause of their occurrence, stand close to those arising from interspecific hybridization.

Occurrence among twin plants from polyembryonic seeds --- Of great interest are the occurrence of haploids among twin plants grown from polyembryonic seeds of rice (RAMIAH et al., 1933, 1935), wheat (NAMIKAWA and KAWAKAMI, 1934, YAMAMOTO, 1936), and cotton (HARLAND, 1936). HARLAND reports that this is not a rare phenomenon in cotton, and explains it in this way: The polyembryonic condition is apparently due to the presence of two embryo sacs in one ovule, and when one of these is fertilized, favorable conditions are created for the development of the unfertilized egg-cell of the neighboring embryo sac. This constitutes another view as to the possible cause of apomictic development of the egg-cell. In essence, it attributes to a developing embryo a stimulating effect upon neighboring embryo sacs. It may possibly be that this explanation is correct, but only for such special cases as polyembryony, when the embryo sacs are directly contiguous, or, perhaps, there are really not two of them, but only one with two egg-cells. In our experiments with *Nicotiana rustica* the development of fertilized ovules had no stimulating effect on neighboring unfertilized ovules in the same ovary.

Induction by low temperature and pollination with X-rayed pollen. --- Lastly, haploid plants have been obtained by subjecting pollinated flowers to low temperature at approximately the time of fertilization, as described by BLAKESLEE, BEILING, FARNHAM and BERGNER (1922) in *Datura stramonium*, and by pollinating emasculated flowers with X-rayed pollen. Haploid plants were first obtained by the latter method by STADLER (1931) in corn, *Zea mays*. Later KATAYAMA

(1934) succeeded in obtaining, by the use of X-rayed pollen, a large number of haploids in *Triticum monococcum*, the frequency of occurrence attaining 17.58 per cent. During the past year two additional papers have appeared on the use of X-rays for obtaining haploids. In the first there is reported the experimental production of a haploid *Triticum dicoccum* and a haploid *Tr. persicum* by pollinating emasculated flowers with X-rayed pollen (YEFERIKIN and VASILYEV, 1936); in the second — of a haploid *Crepis tectorum* (GERASSIMOWA, 1936) by pollinating with normal pollen flowers previously subjected to X-ray treatment. Since the *Crepis* haploid was of pure paternal type with all its recessive (as compared to the mother plant) characters, it is without doubt a case of androgenesis.

Previously there had been reported three cases of generative androgenesis, viz (1) a haploid *Nicotiana Langsdorffii* ($n = 9$) obtained by pollinating an aberrant plant of *N. tabacum* ($2n = 70-72$) with *N. Langsdorffii* ($2n = 18$) pollen (KOSTOFF, 1929), (2) a haploid *N. tabacum* by using pollen of this species on flowers of the synthetic species *N. digluta* -- 24_{II} *tabacum* plus 12_{II} *glutinosa* (CLAUSEN and LAMMERIS, 1929); and, lastly, (3) a haploid *N. sylvestris* by pollinating an *N. tabacum-sylvestris* hybrid ($2n = 36$) with *N. sylvestris* pollen ($2n = 24$) (KOSTOFF, 1934).

The case described by GERASSIMOWA is very clearly one of androgenesis, and the possibility of this phenomenon occurring in flowering plants is thus definitely established. Now there can no longer be the doubt, which persisted after the previously described cases (except that of the *Nicotiana Langsdorffii* haploid), in which the haploids (*N. tabacum* and *N. sylvestris*) might, though with slight probability, have arisen as the result not of androgenesis but of parthenogenesis, because the mother plants contained, in the first case, *tabacum* chromosomes (*N. digluta*) and, in the second, *sylvestris* chromosomes (*N. tabacum-sylvestris* hybrid).

Our own investigations on obtaining haploid plants in *Nicotiana rustica* by the use of X-rayed pollen (begun in 1934 prior to the publication of the papers of KATAYAMA, YEFERIKIN and VASILYEV, and GERASSIMOWA) also proved very successful. In the spring of 1935 we obtained four *N. rustica* haploids as the result of induced parthenogenesis (for detailed description see p. 329).

Conclusions. — From all the foregoing we consider it possible to make the following general conclusions:

1. The egg-cells of the higher flowering plants have preserved the ability, under certain conditions or as a result of certain agencies, to develop by apomixis.

2. In different species it is observed that haploid plants occur with varying frequency, and consequently, the degree of difficulty encountered in inducing experimental parthenogenesis varies

3. The occurrence of haploids in the case of interspecific hybridization (especially of those crosses in which it is exceptionally difficult to obtain hybrids), or of the use of X-rayed pollen, or of subjection to low temperature at the time of fertilization, is induced, in all probability, by the penetration into the embryo sacs of such male gametes as prove incapable of fusing with the nucleus of the egg-cell or realize such fusion very rarely ¹⁾.

Stimulation of egg-cells to development by pollen tubes alone without the participation of generative cells seems very doubtful

Morphology of haploids

With respect to their morphological characters, almost all the haploids so far reported constitute reduced replicas of the parental plants - maternal reduced type, in the case of generative parthenogenesis, and paternal, in case of androgenesis (see Summary Table, column 3). The only exceptions are the *Triticum compactum* and *Tr vulgare* haploids, which were absolutely identical to the maternal plants, differing only by their less well-developed generative organs and their high sterility. The *Nicotiana rustica* haploids we obtained also show great similarity to the mother plants, but differ from them by their much smaller leaves and flowers and lower vigor. As regards height, however, the *N. rustica* haploids differ scarcely at all from diploids. Similarly, the *Gossypium Davidsonii* haploid (SKOVSTED, 1935) was of the same height as the diploid plants, but had smaller leaves and shorter branches.

¹⁾ This hypothesis is confirmed, as far as obtaining haploids by the use of X-rayed pollen is concerned, by the observations of ПОДУБНАЯ-АРНОЛДИ (1936) and by our own on the formation of generative nuclei in the tubes of X-rayed pollen, which have shown that the nuclear divisions are irregular, resulting in the formation, of gametes which have incomplete chromosome sets and which are, most probably, inviable

Reduction in the chromosome number in somatic cells, as we see, is externally reflected, first of all, in the smaller size of the plant's organs. However, there does not always occur a mere mechanical reduction in size. Thus, many investigators have noted the appearance of new characters resulting from the haploid condition. The *Crepis capillaris* haploids (HOLLINGSHEAD, 1930) were characterized by a very marked change in the shape of the rosette leaves (the margins were less deeply indented) and a unique branching habit. The *Pharbitis Nil* haploid (U, 1932) had leaves somewhat altered in contour and a corolla markedly dissimilar in shape as well as size, being pentagonal (five-rayed) instead of round.

A very marked change in morphological characters in connection with the haploid state has been described by YEFERIKIN and VASILYEV (1936) in the case of *Triticum dicoccum* and *Tr. persicum* haploids, and also by VASILYEV (1936) in that of a *Tr. durum* haploid. In these haploids the external appearance was so greatly altered that the investigators found the haploids showed greater resemblance to *Tr. monococcum* than to the parental species.

The *Nicotiana rustica* haploids did not exhibit marked variations, but the one haploid plant of the variety *humilis* was characterized by longer, slimmer leaves than those of the corresponding diploids.

The examples cited indicate that the reduction in chromosome number is not always externally reflected only in decreased size of organs but also is often connected with a somewhat different manifestation of the genom in the formation of characters.

Decrease in the size of cells both in somatic and sporogenous tissues is characteristic of all haploid plants. Data on cell size in the haploids obtained by a number of investigators are given in Table 19 (page 359).

There appears to be a definite correlation between reduced chromosome number and cell size. Most data (including our own measurements of cells and nuclei in the meristem of *N. rustica* haploids) show that halving the chromosome number results in approximately halving the size of the cells. The data of KOSTOFF constitute an exception. KOSTOFF found that in haploid *Nicotiana Langsdorffii* the size of cells is reduced not to one-half but to one-quarter the diploid size. Measurements of cells in different tissues of the *Oenothera rubricalyx* haploid (GATES and GOODWIN, 1930) revealed some variation in this correlation as among the different organs.

TABLE 19. SIZE OF CELLS AND NUCLEI IN HAPLOID PLANTS AS COMPARED WITH DIPLOID PLANTS

Species	Meristem of root-tips		Size of cells of epidermis	Size of PMC's	Size of normal pollen grains	Investigators
	Size of cells	Size of nuclei				
	(ratio of size in n to that in 2n plants)					
<i>Datura stramonium</i>				1 2	1 1	BELLING and BLAKESLEE
<i>Nicotiana Langsdorffii</i>	1 4					KOSTOFF
<i>Nicotiana rustica</i>	about 1 2	1 2			1 1	IVANOV
<i>Oenothera franciscana</i>		1 2 4				EMERSON
<i>Oenothera rubricalyx</i>		1:3 2	1 2 3 (1.3 5)	1 2 1		GATES and GOODWIN
<i>Matthiola incana</i>				about 1 2		LESLEY and FROST
<i>Lycopersicum esculentum</i>		1 1 64		1.1 9	1 1	HUMPHREY, LINDSTROM and KOOS
<i>Portulaca grandiflora</i>					1 1	OKURA
<i>Pharbitis Nil</i>				1 2.2		U

The occurrence of diploid cells in haploid tissue and even of diploid organs in haploid plants has been noted by a number of investigators (Table 20).

These observations on the occurrence of diploidy in haploid plants are of particular interest, because in work with haploids there is considerable likelihood that diploid shoots may be obtained, which by self-pollination will be normally fertile and the progeny obtained therefrom be characterized by exceptional homozygosity.

TABLE 20. FREQUENCY OF OCCURRENCE OF DIPLOIDY IN HAPLOID PLANTS

Haploids	Diploid shoots	Root-tips		Haploid root-tips with diploid areas	PMC's with 2n chromosomes	Investigators
		2n	n			
<i>Nicotiana glutinosa</i>		From 0 to 55.6% aver. 22.6%		2.21%	+	WEBBER
<i>Nicotiana Langsdorffii</i>		1	58	+		KOSTOFF
<i>Nicotiana tabacum</i>		22	52	8	+	RUTTIE
<i>Nicotiana sylvestris</i>				+		KOSTOFF
<i>Lycopersicum esculentum</i>				+		LINDSTROM and KOOS
<i>Crepis capillaris</i>	1			+		HOLLINGSHEAD
<i>Crepis tectorum</i>	1			+		GERASSIMOWA
<i>Oryza sativa</i>	+			+		MORINAGA and FUKUSHIMA
<i>Pharbitis Nil</i>					+	U.
<i>Nicotiana rustica</i> var. <i>humilis</i>				2.56%		IVANOV
<i>Nicotiana rustica</i> var. <i>texana</i>				2.24%		IVANOV

Diploid cells may develop, as the organs grow, into diploid areas, the cells of which are considerably larger than haploid cells (GERASSIMOWA, 1936). The number of diploid cells in haploid tissues shows, according to WEBBER (1933), a rapid increase with the growth of the plant. Thus, he found that in an *N. glutinosa* haploid at the age of 24 weeks only 5.7 per cent of the root-tips were diploid (at an earlier age none were found), while at the age of 37 weeks 55.56 per cent of the tips were diploid.

Doubling of the chromosome set in haploids is, apparently, realized with considerably greater ease than in diploids.

Microsporogenesis in haploids

General character of meiosis in haploids. --- The formation of microspores in all the haploids so far known is extremely irregular. At meiosis all the chromosomes in the nuclei of pollen mother-cells (PMC's) usually remain univalents. This is the cause of the chief irregularities, especially of the heterotypic division. The entire absence of homologous pairs of chromosomes in real haploids derived from diploid species, or the partial homology of chromosomes of haploids derived from amphidiploid or autopolyploid species, results in chromosome conjugation being completely absent or occurring only rarely. The univalent chromosomes are usually not disposed in orderly fashion with respect to the equatorial region of the cell, do not form regular equatorial plates, and at the first division are scattered haphazard over the spindle. At first anaphase they pass entirely at random to the poles and form there groups varying in number. As a result, after the ensuing homeotypic division has been passed through with comparative regularity, microspores are formed which have nuclei with incomplete sets of chromosomes and which are, consequently, for the most part inviable. This is, in brief outline, the general course of meiosis in most haploids. However, this is only the general course, various deviations from which may occur in many PMC's even in one and the same haploid plant.

Univalent chromosomes outside the spindle area at the first division.

-- At the heterotypic division univalent chromosomes are often found outside the spindle area. In the *Nicotiana rustica* haploids we counted from 0 to 9 chromosomes lying in the plasma outside the spindle area, the frequency of occurrence of PMC's with such chromosomes amounting to 33.3 per cent. In many haploids of other species univalents have also been found outside the spindle area at the first division, but, unfortunately, counts were not made in all cases. Thus, this phenomenon was noted quite frequently in the following haploids: *Datura*, *Nicotiana tabacum* (14.6%), *N. glutinosa* (14.08%), *N. Langsdorffii*, *N. sylvestris*, *Portulaca grandiflora* and *Matthiola incana*; and less frequently in the following: *Oenothera (franciscana, Hookeri, blandina)*, *Pharbitis Nil*, *Crepis capillaris*, and *Solanum nigrum*.

This lack of any orderly disposition of the chromosomes at the

heterotypic division complicates its course, making it deviate from the normal. The univalents, scattered over the spindle, are distributed to the poles entirely at random. The chromosomes lying outside the spindle area do not participate in this distribution, but form supplementary groups, which subsequently passing through the second division, produce micronuclei.

Division of univalents at first anaphase. At first anaphase the univalent chromosomes do not always pass to the poles as wholes. In this respect a great difference is noted among haploids of different species. On the one hand, division of univalents at first anaphase, followed by separation of the daughter halves to opposite poles, has been observed in single instances, as a rare exception, in the following haploids: *Nicotiana Langsdorffii*, *N. tabacum*, *Pharbitis Nil*, *Crepis capillaris*, *Triticum compactum*, *Tr. vulgare*, *Portulaca grandiflora*, and *Solanum nigrum*. In the *N. rustica* haploids division of the univalents was also exceptionally rare. On the other hand, cases of more frequent division have been reported. For example, the *Matthiola incana* hybrids exhibit very frequent division of univalents. In their case LESLEY and FROST (1928) report that division of all univalents at the heterotypic anaphase was the usual mode of behavior, being observed in 70 per cent of the PMC's. In *Oenothera* haploids division of univalents at heterotypic anaphase, though not so common, was also observed quite often.

Presumably the frequency of occurrence of the division of univalents at the heterotypic division is determined by the specific peculiarities of the haploids, since it differs in different species. But it is also possible that it depends on the conditions under which the haploids arose. For example, the various *Nicotiana tabacum* haploids, obtained in considerable numbers by different investigators, do not exhibit the same frequency of division of univalents. CHIPMAN and GOODSPEED (1927) give in their paper a table showing the frequency of division of univalents at first anaphase -- of 80 PMC's examined, 28 exhibited division of from 1 to 8 univalents and 4 division of all 24 univalents. Other investigators, however, either did not observe such division at all in the *tabacum* haploids they studied or report it as of very rare occurrence (CLAUSEN and JAMMERTS, 1929; McCRAE, 1932; and others).

Non-reduction and random distribution of univalents at the first

division. — The behavior of the chromosomes at the heterotypic division is decisive as regards the formation of viable spores. Haploid plants are always characterized by a large amount of abortive pollen. Almost always they are practically sterile. This is understandable, since with random distribution of the univalent chromosomes to the poles a full set of chromosomes in the daughter nuclei will be an extremely rare occurrence, the number of chromosomes distributed to either pole ranging from 0 to n . The probability of the realization of a chance distribution of chromosomes at the heterotypic division of the 0- n type — no chromosomes at one pole and all (n) chromosomes at the other — may be calculated, knowing the number of univalents. It may be expressed in the form of the ratio between the frequency of occurrence of the two extremes of the frequency distribution from 0 to n and the sum of the frequencies of all the other members of the series $2 - 2^n - 2$ or, reducing, $1 \cdot \frac{2^n - 2}{2}$.

If one compares the theoretical probability of the occurrence of a 0- n type of distribution with the observed frequency of occurrence of PMC's exhibiting this type at the heterotypic division in haploids, there is revealed a great discrepancy. Unfortunately, all investigators have not made the necessary counts, and even those that have done so have limited themselves to a small number of counts. However, for the purpose of illustrating the discrepancy between expected and actual results the data given in Table 21, for several haploids with different chromosome numbers, will suffice:

TABLE 21

Haploids	Chrom no	Frequency of 0—n distribution		Investigators
		Expected	Observed	
<i>Triticum monococcum</i>	7	1:63	6:500 or 1:83.3	KATAYAMA, 1935
<i>Oenothera Hookeri</i>	7	1:63	1:53	BUTLER, 1933
<i>Oenothera franciscana</i>	7	1:63	14:244 or 1:17.4	EMERSON, 1929
<i>Oenothera blandina</i>	7	1:63	2:110 or 1:55	CATCHESIDE, 1932
<i>Nicotiana glutinosa</i>	12	1:2,047	115:3,291 or 1:28.6	WEBBER, 1933
<i>Triticum vulgare</i>	21	1:1,048,575	2:300 or 1:150	YAMAMOTO, 1936
<i>Nicotiana rustica</i>	24	1:8,388,607	24:596 or 1:24.8	IVANOV (present investigation)

From these data it may be concluded that the observed formation of equatorial plates containing all univalent chromosomes in some PMC's, at the same time as nuclei of neighboring PMC's from the same anther are at first anaphase or beginning of second metaphase, is not due only to random chromosome distribution. If this were the only cause, the probable frequency of occurrence of the 0—n type of distribution would be 1 : 2,047 for 12 univalents, as in haploid *Nicotiana glutinosa*, and for 24 univalents, as in haploid *N. rustica*, it would decrease to such an extent as to amount practically to complete absence of the type, since it would occur only one time out of 8,388,607.

Actual observations of chromosome distributions in PMC's of *Nicotiana glutinosa* revealed that cells, in which all the chromosomes are concentrated in one group and preparing for the second division, are not of rare occurrence, being found in one case out of 28.6. Similar data obtained by us for *N. rustica* show one case out of 24.8 (and not out of 8 million). It seems perfectly clear to us that the fairly frequent occurrence of cells with a 0—n chromosome distribution is the result of non-reduction, i.e., of the omission by these cells of the heterotypic division and the beginning of the homeotypic division.

Homeotypic division. — The second division in haploid plants usually proceeds regularly. All the chromosomes, having formed groups at the poles, divide, and their halves are distributed to the poles of their respective spindles and form nuclei of the "tetrad"

stage. In those cells, in which univalent chromosomes remained outside the spindle at the first division, a chaotic condition is observed, since additional small spindles are formed and a division of many groups occurs. As a result, micronuclei are produced. Due to the division of univalents at the first division, there may be observed at the homeotypic anaphase lagging chromosomes, or rather halves of chromosomes.

Sporads of haploid plants. - The formation of cells at the "tetrad" stage in all haploid plants, without exception, is extremely irregular. In all haploids the number of cells per sporad varies from one to more than four (to as many as nine). Monads, diads, triads, tetrads, pentads, and so on, are formed. Data on the number of cells formed after cytokinesis by PMC's of various haploids are given in Table 22 (page 366). Tetrads and diads occur with the greatest frequency. As we have already mentioned in the case of *Nicotiana rustica*, the number of nuclei frequently exceeds the number of cells, i.e., some cells may have several nuclei differing in size.

The term "tetrads", widely used in biological literature to designate the groups of cells formed as a result of the meiotic division of PMC's, as applied to haploids (and also to a large number of interspecific hybrids) is very inept, since these groups, as has already been noted, do not always consist of four cells. It seems to us more appropriate to use the term "sporads" proposed by WEBBER (1933). We have, therefore, used this term in describing meiosis in *N. rustica* haploids, using the term "tetrads" only for those sporads which consist of four cells.

General features of microsporogenesis in haploids. The general features of microsporogenesis in haploid plants arising from diploid and allopolyploid species are: absence of chromosome conjugation, random distribution of the univalents to the poles at first anaphase, frequent occurrence of irregularly scattered chromosomes outside the heterotypic spindle, infrequent division (except for the *Matthiola incana* haploids) of the univalent chromosomes at first anaphase, and comparatively frequent cases of non-reduction. As a result of such irregularities, spore nuclei are formed with incomplete chromosome sets. Cytokinesis proceeds irregularly, monads, diads, triads, tetrads, pentads, hexads, etc. being formed.

Most of the microspores are inviable and perish at an early stage of

TABLE 22. NUMBER OF CELLS IN SPORADS OF HAPLOIDS AFTER CYTOKINESIS

Haploids	Number of cells in sporads					Investigators
	1	2	3	4	over 4	
<i>Crepis capillaris</i>	12	195	57	19	-	HOLLINGSHEAD
<i>Datura stramonium</i>	—	187	a few	1385	278	BELLING and BLAKESLEE
<i>Datura stramonium</i>	—	1160	-	2840	-	LESLEY and FROST
<i>Mathiola incana</i>	3	285	99	19	-	WEBBER
<i>Nicotiana glutinosa</i>	—	48	18	456	116	KOSTOFF
<i>Nicotiana Langsdorffii</i>	+	+	+	+	+	CHIPMAN and GOODSPEED
<i>Nicotiana tabacum</i>	-	18	34	692	43	IVANOV
<i>Nicotiana rustica</i>	4.9%	29.6%	19.8%	38.3%	7.4%	CATCHESIDE
<i>Oenothera blandina</i>	1	63	21	43	2	BLEIER
<i>Oenothera franciscana</i>	—	73	16	104	44	BLEIER
<i>Oenothera Hookeri</i>	-	13	3	156	14	U. KATAYAMA
<i>Pharbitis Nil</i>	+	+	+	+	+	LINDSTROM and KOOS
<i>Lycopersicum esculentum</i>	-	-	+	+	+	YAMASAKI
<i>Triticum compactum</i>	+	+	-	+	+	KAMIAH <i>et al</i>
<i>Oryza sativa</i>	2	24	33	41	-	

development. A viable microspore is obtained only in case the nucleus receives the entire haploid complex of chromosomes. This may be realized by a chance distribution of the univalents according to the type 0-n, or, which apparently occurs more frequently in haploids with a large chromosome number, as a result of non-reduction, *i.e.*, the omission by all the univalents of the first division, which is replaced by the homeotypic division. This assumption is confirmed by the comparatively close coincidence of the percentage of viable pollen and the percentage of microspores formed in diads.

Instances of chromosome conjugation in haploids. --- Despite the fact that absence of chromosome conjugation is a characteristic feature of the meiosis of haploids arising from diploid and allopolyploid species, the pairing of chromosomes has been reported for many haploids, the frequency of occurrence varying. Inasmuch as such chromosome

behavior is characteristic of whole groups of haploids and is significant from several points of view, it seems advisable to consider this phenomenon more in detail.

In the first place, are these chromosome pairs real bivalents? Undoubtedly, in the case of the *Oenothera* haploids. CATCHESIDE (1932) established the presence of such pairs in an *Oenothera* haploid at prophase and at diakinesis, as well as at later stages. This investigator also studied in *O. blandina* the chiasma types of the bivalent, trivalent, and quadrivalent chromosomes, which he found in 20 per cent of the PMC's, the other 80 per cent having only univalents. Real chromosome conjugation has also been established for other *Oenothera* haploids (EMERSON, 1929; STOMPS, 1930, 1931; BLEIER, 1933). In all other haploids in which pairs have been observed at prophases and diakinesis it may be a rare, chance phenomenon or, perhaps, a case of secondary association. KIHARA, for instance, has proposed that such pairs of non-homologous chromosomes be called "bipartites".

As regards the formation of "bivalents", haploids (other than the *Oenothera* haploids) may be divided into those not forming any at all, all chromosomes in all PMC's being univalents, and haploids having one or two "bivalents" (or bipartites) in occasional PMC's.

It is quite a different matter when the haploids have arisen from species having homologous chromosomes in their haploid complex, i.e., from autopolyploid species or allopolyploid species having chromosomes of closely related species. In such cases chromosome conjugation at meiosis of haploids is of frequent occurrence. The *Solanum nigrum* haploids (JØRGENSEN, 1928) are in this respect the most constant, forming at meiosis in most PMC's 12 bivalents and 12 univalents.

The formation of bivalents, trivalents, etc. in haploids from tetraploid and hexaploid species does not, of course, always embrace all the chromosomes, since it is evident that in the course of the evolutionary process the chromosomes in the sets of these species which at the genesis of the species were identical have changed not only in their genic constitution but probably also in the way of the formation of non-homologous parts (crossing over, translocations, inversions, deletions, etc.). Thus, in haploids from hexaploid wheats, *Triticum vulgare* and *Tr. compactum*, YAMASAKI (1936) observed at

TABLE 23. FORMATION OF "BIVALENTS" IN HAPLOIDS

Haploids	Chromosome no	Heterotypic division			Investigators
		All chromosomes univalent	One "bivalent"	Two "bivalents"	
<i>Datura stramonium</i>	12	all PMC's			BELLING & BLAKESLEE; and others
<i>Matthiola incana</i>	7 + frag	all PMC's			LESLEY and FROST
<i>Nicotiana glutinosa</i>	12	all PMC's			WEBBER
<i>Nicotiana Langsdorffii</i>	9	as a rule	rarely		KOSTOFF
<i>Nicotiana sylvestris</i>	12	as a rule	rarely		KOSTOFF
<i>Oenothera franciscana</i>	7	2,187	143	3	BLEIER
<i>Oenothera Hookeri</i>	7	1,121	145	9	BLEIER
<i>Pharbitis Nil</i>	15	all PMC's			U; KATAYAMA
<i>Lycopersicon esculentum</i>	12	all PMC's			LINDSTROM and KOOS
<i>Crepis capillaris</i>	3	all PMC's			HOLLINGSHEAD
<i>Oryza sativa</i>	12	as a rule	rarely	occasionally	MORINAGA and FUKUSHIMA
<i>Triticum monococcum</i>	7	490	10		KATAYAMA
<i>Nicotiana tabacum</i> ¹⁾	24	as a rule	rarely		CHIPMAN and GOODSPEED
<i>Nicotiana rustica</i>	24	947	10		IVANOV
<i>Portulaca grandiflora</i>	9	all PMC's			OKURA
<i>Zea mays</i>	10	all PMC's			RANDOLPH
<i>Oenothera blandina</i> ²⁾	7	938	216	17	CATCHESIDE

¹⁾ Entirely different data as regards chromosome conjugation in haploid *N. tabacum* have been reported by S. P. HACHATUROV, who gives chromosome counts at first metaphase indicating that cases where all 24 chromosomes are univalents occur rarely. Bivalents -- ranging in number from 1 to 12 -- were observed, on the other hand, in 192 out of 198 cases (HACHATUROV, 1937)

²⁾ At meiosis in the *O. blandina* hybrids not only bivalents but also trivalents and quadrivalents were observed (CATCHESIDE, 1932)

meiosis from 0 to 4 bivalents and occasional trivalents. Out of 964 PMC's examined 460 had $21_1 + 0_{II}$, 353 $-19_1 + 1_{III}$, 116 $-17_1 + 2_{III}$, 22 $-15_1 + 3_{III}$, 4 $-13_1 + 4_{III}$, 5 $-18_1 + 1_{III}$, 3 $-16_1 + 1_{II} + 1_{III}$, and, lastly, one had $14_1 + 2_{II} + 1_{III}$. In other words, all the chromosomes remained univalents (21_1) in 47.7 per cent of the PMC's, while bivalents and trivalents were found in 52.3 per cent. It is also of interest that there were never seven bivalents (the basic chromosome number of *Triticum*) but always less (not more than 4), and that of 499 cells having bivalents 356 had only one pair.

The *Brassica Napella* haploids (MORINAGA and FUKUSHIMA, 1933) constitute a good example of chromosome behavior at meiosis in a haploid from an allopolyploid species with similar genomes. As was established by MORINAGA (1929), *Brassica Napella* is a bi-genomic species, having a genome "a" (10 chromosomes) and a genome "c" (9 chromosomes). MORINAGA assumes, therefore, that *B. Napella* is a tetraploid from a cross between two *Brassica* species - one with $n = 10$ and the other with $n = 9$. In *B. Napella* haploids, occurring spontaneously in field cultures, at the heterotypic division there are from 0 to 6 or 7 bivalents, an entire absence of bivalents occurring rarely.

In order to make our survey complete, it is necessary to mention a few "haploids" obtained by self-pollination of polyploids or by crossing them with the parental plants. As a result of generative parthenogenesis there arose "haploid" plants of *Oenothera Lamarckiana gigas* ($2n = 28$), an autotetraploid from *O. Lamarckiana* (HAKANSON, 1926), and of *O. biennis gigas* ($2n = 28$) (STOMPS, 1928). Actually there were obtained diploid *O. Lamarckiana* and *O. biennis*, but they were "haploid" as compared to the mother plants. Meiosis proceeded in identical manner to that of diploids.

By crossing an amphidiploid plant, *Nicotiana rustica-paniculata* ($2n = 72$), with the parent species there arose parthenogenetically an *N. rustica-paniculata* ($2n = 36$), haploid as compared to the mother plant. Meiosis in this "haploid" was typical for hybrids between *N. rustica* and *N. paniculata*.

Other haploids, similar to the afore-mentioned, have been reported for *Digitalis mertonensis* (BUXTON and DARLINGTON, 1932) and for *Aegilotriticum* (KATAYAMA, 1935). Meiosis in such plants, obtained as a result of haploid parthenogenesis, does not exhibit any unique

features, being identical to that of the plants which gave rise to the polyploid mother plants.

Megasporogenesis in haploids

A study of megasporogenesis in plants presents considerably greater difficulty than a study of microsporogenesis, since it is very difficult to find for fixation ovarian tissue at the stage of meiotic division of the egg mother-cells (EMC's) or at the stage of development of female gametophytes. This is apparently the reason why so few of the papers on haploid plants give data on the meiosis of EMC's, the formation of megaspores, and the development of embryo sacs. Actually we have only two detailed descriptions, viz., that in WEBBER's paper (1933) on haploid *Nicotiana glutinosa* and that in the paper of two Japanese investigators (MORINAGA and FUKUSHIMA, 1934) on haploid rice. With the exception of the authors of these two papers, all the other investigators merely mention that meiosis of the EMC's is identical to that of the PMC's (CHIPMAN and GOODSPEED, 1927; GAINES and AASE, 1926; and others).

WEBBER describes megasporogenesis in the *Nicotiana glutinosa* haploids approximately as follows: Up to the second telophase meiotic behavior of chromosomes in EMC's and PMC's is practically identical. However, the distribution of the chromosomes to the poles at first anaphase is somewhat more regular. Only very few EMC's develop beyond the second telophase. Usually following the latter phase their disintegration sets in, and they are supplanted by the surrounding somatic cells. The occasional megasporads which develop usually contain two spores, the nuclei of which are approximately the same size as those of microdiads and contain, on the basis of rough counts, about 12 chromosomes. The early stages of development of the female gametophyte proceed very rapidly. From 4 to 7 nuclei are formed, which, in the majority of cases, form a single compact group and sometimes fuse, forming giant nuclei. Usually at the time when fecundation should take place such embryo sacs degenerate. Only very rarely are embryo sacs encountered which are close to normal.

According to MORINAGA and FUKUSHIMA, in haploid rice (*Oryza sativa*) meiosis in EMC's proceeds exactly the same as in PMC's. At diakinesis there are observed only 12 univalent chromosomes. At the

heterotypic division they are distributed at random to the poles. Examples are given of such random distributions, among which is the 0-12 type. Following the formation of the two daughter nuclei a cell wall is formed separating the two megaspores. Both megaspores usually begin homeotypic division at the same time, as a result of which four megaspores are formed. In the majority of ovules of haploid rice degeneration then sets in, or it sets in even earlier, during the homeotypic division. In only a comparatively few ovaries does the formation of embryo sacs proceed further. In such cases one of the spores usually enlarges, its nucleus divides three times, and a normal, octonucleate embryo sac is formed.

As is seen from these descriptions, in haploids most of the megaspores are inviable and perish at an early stage of development. Only a very few of them are able to develop into embryo sacs. The latter, in haploid *Nicotiana glutinosa*, are never fully normal, and usually various irregularities occur which result in their disintegration. In haploid rice, on the other hand, embryo sacs develop only from viable megaspores and are normal. Our own observations of megasporogenesis in *Nicotiana rustica* haploids, described in detail above, show that the heterotypic division of EMC's proceeds in practically the same fashion as that of PMC's. As a result of random distribution of univalent chromosomes, there are very often formed, not two cells as in diploid *N. rustica*, but more. At this stage the megaspores degenerate; possibly their degeneration sets in immediately after the second division. Only very rarely is it possible to observe the development of an embryo sac. In such cases their formation proceeds very normally: after three successive nuclear divisions an octo-nucleate embryo sac is formed. We did not observe the degeneration of any embryo sacs. Apparently, embryo sacs are formed only from spores possessing a full haploid set of chromosomes.

From the cases surveyed it is clear that haploid plants produce a very small number of ovules capable of being fecundated, just as their anthers contain only an insignificant number of viable microspores.

The progeny of haploids

The progeny of haploid plants are of great interest in connection

with the question of utilizing haploids for breeding purposes. The high degree of sterility of the pollen grains, as also of the ovules, as described in papers on haploid plants, results in haploids having very few progeny. In the case of some haploids progeny were obtained only after preliminary reproduction by cuttings, *i.e.*, only by increasing the number of haploid individuals

Knowing the peculiarities of meiosis in PMC's of haploids, it is clear that viable gametes, having full haploid, diploid, or tetraploid chromosome sets, may arise only in the following exceptional cases:

1. As the result of a chance 0-n distribution of univalents at the first division, followed by a regular second division and the formation of diads with haploid nuclei. This case occurs in all haploids which have been sufficiently well studied.

2. As the result of non-reduction, *i.e.*, the omission of the heterotypic division by all the univalent chromosomes, followed by a regular second division and the formation of diads with normal haploid nuclei. This phenomenon has been observed in the following haploids: *Datura* (up to 10–20%); especially often (up to 70%) in *Matthiola incana*, *Crepis capillaris*, *Nicotiana tabacum*, and *N. rustica* (the present investigation); more rarely in *N. glutinosa*, *N. Langsdorffii*, *N. sylvestris*, *Oenothera*, *Pharbitis Nil*, *Lycopersicum esculentum*, and *Oryza sativa*.

3. A very rare case, reported by BLEIER (1933), in which the univalents divide both in the first and second division and distribution is regular among the four nuclei, resulting in the formation of tetrads with haploid nuclei.

4. The formation of monads at the close of the first division, the univalents not having disjoined during this division and there being no homeotypic division. Monads with haploid nuclei are formed.

5. As a result of non-reduction, the univalents dividing at second metaphase but not separating to the poles. Monads with 2n nuclei are formed.

6. A case similar to (3), but at the second division the half-chromosomes fail to separate to the poles and are included in one common nucleus. As a result, diads with 2n nuclei are formed.

7. A case like (1) or (2), but with non-disjunction of the half-chromosomes, which form one common nucleus. Monads with 2n nuclei are formed.

In addition, the formation of viable spores is possible in the case of the division of diploid and binucleate PMC's. Such mother-cells have been revealed in the sporogenous tissue of a number of haploids, e.g., *Nicotiana tabacum* (RUTTLE, 1928), *N. glutinosa* (WEBBER, 1933), *Oenothera* haploids (BLEIER, 1933), *Crepis capillaris* (HOLLINGSHEAD, 1930), *Pharbitis Nil* (U., 1932), *Triticum compactum* (GAINES and AASE, 1926), and also in the *Nicotiana rustica* haploids obtained by us. In such cases spores may be found with $1n$, $2n$, or $4n$ nuclei.

In the majority of cases the progeny of haploids are diploids, normally fertile, and identical to the initial diploid plant from which the haploid arose. Only very rarely do haploid plants appear. In all the following cases plants grown from seeds of haploids all proved to be diploid and uniformly homozygous; *Lycopersicum esculentum* (LINDSTROM and KOOS, 1931; HUMPHREY, 1934), *Nicotiana tabacum* (CHIPMAN and GOODSPEED, 1927), *Oryza sativa* (MORINAGA and FUKUSHIMA, 1934), *Portulaca grandiflora* (OKURA, 1933), and *Solanum nigrum* (JØRGENSEN, 1928). Particularly complete homozygosity has been observed in the case of the progeny of tomato haploids, which have been propagated for five or six generations and have maintained definite varietal characters.

The progeny of the following haploids consisted mostly of diploid plants, but included a very few haploid plants: *Oenothera blandina* (CATCHESIDE, 1932), *O. franciscana* (DAVIS and KULKARNI, 1930), and *Triticum monococcum* (KATAYAMA, 1935).

In the *Datura stramonium* haploids (BLAKESLEE, MORRISON, and AVERY, 1927), however, in the numerous progeny of vegetatively propagated haploids, which also consisted chiefly of diploid plants, there were found a few triploids, tetraploids, trisomic mutants, and cases of gene mutations. Trisomic mutants were also found among the progeny of an *Oenothera franciscana* haploid (ANDERSON, 1933).

From our *Nicotiana rustica* haploids we, too, succeeded in obtaining seeds, which were sown in the summer of 1936. The plants obtained were, for the most part, diploids, but among them there were found one triploid ($2n=72$) and several other evidently polyploid plants, the chromosome numbers of which have not yet been precisely determined.

As is clear from the foregoing, haploid plants may be used for the purpose of obtaining diploid plants which will, in most cases, be

homozygous. But homozygosity in plants propagated by seed can scarcely be attained in the progeny of those haploids which at meiosis have bivalents or bipartites. In such cases, evidently, an exchange of parts of chromosomes is possible, and, hence, the formation of gametes differing in genic constitution, although they may have a full haploid set of chromosomes. Moreover, from the work of BLAKESLEE, MORRISON, and AVERY (1927) it may be concluded that the mutability of individual genes, probably due to the haploid state, is greatly increased, which also results in the formation of dissimilar gametes. All this should be kept in mind when studying haploids from the viewpoint of the requirements of practical breeding work. The greater mutability of haploids and also the occurrence of polyploid forms among their progeny may prove useful in breeding work. However, these factors make it impossible always to speak with assurance regarding the production of absolutely homozygous strains by using the seeds of haploids [as NAVASHIN recommended *Semcnovodstvo* ("Seed-Growing"), 1934, No. 2].

Methods of artificially inducing polyploidy by decapitating young plants have been quite thoroughly elaborated during recent years (JØRGENSEN, KARPECHENKO, SHCHAVINSKAYA, and others), and they may play in this connection a very great rôle. In this way undesirable consequences of chromosome conjugation at meiosis can be entirely averted, but the increased mutability of the haploids can scarcely be prevented, since mutations may arise in somatic cells also. However, diploids obtained by decapitation reflect gene changes at once in the homozygous state, not in the heterozygous state, as is the case when the plants are propagated by seed.

Classification of haploids

Although at the present time the number of haploid plants is not sufficiently great to enable one to make an exact classification, it is possible to place them tentatively in definite groups, thus facilitating an understanding of their chief peculiarities. Inasmuch as the most important process in the life of a plant is that of reproduction, involving the formation of viable gametes, it seems to us that the character of the meiotic behavior of the chromosomes of a haploid should constitute one of the chief criteria in classification. Using this

as a basis, BLEIER (1933) already made an attempt to classify haploids. But at that time considerably fewer haploid plants were known, and, consequently, he placed in a single group (*Datura* type) haploids as widely different in their chromosome behavior as the *Datura* haploids, having at the heterotypic division only univalent chromosomes, and the *Oenothera blandina* haploids, at meiosis of which bivalents are formed in approximately 18-19 per cent of the PMC's. Similarly, in this group are included the *Matthiola incana* haploids, which differ markedly from the others, both as regards chromosome behavior at the heterotypic division (70% non-reduction) and as regards chromosomal constitution (7 + frag.).

A second basis for classification, also fundamentally quite correct, was advanced by KATAYAMA (1935a), viz., the chromosome complex. KATAYAMA divides all haploids into *monohaploids* (those arising from diploid or "basal" species) and *polyhaploids* (those arising from polyploid species). Each of these categories is subdivided into *euhaploids*, if the genomes (or genom) making up the chromosome complex are complete in their components, *heterohaploids*, if certain chromosomes or parts of chromosomes in any of the genomes are duplicated or eliminated; and, lastly, *pseudohaploids*, those arising from autopolyploids or autoallopolyploids and which in reality are diploids.

This scheme of classification is not only fundamentally correct but quite convenient, but still it does not make it possible to group haploids so that within each group they should have a more or less similar type of meiosis. Again *Datura stramonium* and *Oenothera* haploids fall in one group, the monohaploids, while the polyhaploid group (AB) embraces *Nicotiana tabacum* (or *N. rustica*) haploids, not having hardly any chromosome conjugation at meiosis, and *Brassica Napella* haploids, forming at meiosis, as a rule, from 1 to 6 or 7 bivalents.

On the basis of our detailed analysis of meiosis in all haploids so far reported, we propose the following classification based on the genomic constitution of the haploids and the type of meiotic behavior:

First of all we divide the haploids into monohaploids and polyhaploids. Under monohaploids we include (as KATAYAMA does) all haploids originating from diploid or "basal" species. Their chromosome complex (A) is made up of non-homologous chromosomes. Among the monohaploids we distinguish four types:

1) *Datura* type — characterized by complete absence of bivalents or bipartites at meiosis, all chromosomes being univalents;

2) *Oryza* type — chromosomes, as a rule are all univalents, but sometimes one or two "bivalents" or bipartites are encountered in occasional PMC's;

3) *Oenothera* type — in the majority of PMC's all the chromosomes are univalents, but bivalents (from 1 to 3) are of frequent occurrence and occasional trivalents and quadrivalents are sometimes encountered;

4) *Matthiola* type — characterized by the presence of a fragment in addition to the haploid chromosome complex and by the absence of chromosome conjugation at meiosis, particularly by the omission of the heterotypic division in most PMC's and by the formation of diads. The latter feature we consider more fundamental as a distinguishing characteristic of this type than the presence of the fragment, since as a result of non-reduction a large number of viable microspores are formed.

The polyhaploids we subdivide into *allohaploids*, i.e., haploids having haploid chromosome sets from different species (haploids arising from allopolyploid species), and *pseudohaploids*, those having several haploid sets from one and the same species (haploids arising from autopolyploids). Strictly speaking, the forms in the latter group are not real haploids but rather diploids, triploids, etc., but their origin is connected with generative parthenogenesis (or androgenesis) in an autopolyploid.

The allohaploids are, in turn, divided into five types:

1) *Nicotiana* type --- having two genomes from two different species (AB); at meiosis, as a rule, all chromosomes are univalents; bivalents are observed rarely in occasional PMC's;

2) *Brassica* type — having two genomes (AB) but so closely related that chromosome conjugation (although not complete) is observed in most PMC's; however, in occasional PMC's all chromosomes may be univalents;

3) *Solanum* type -- having three genomes, two of which are identical (AAB) or very closely related; meiosis approximates the type $A_{II} + B_I$;

4) *Triticum* type --- having three different genomes (ABC); at meiosis bivalents and trivalents are of frequent occurrence;

5) *Aegilotricum* type --- having four different genomes (ABCD); at meiosis most PMC's contain only univalents, chromosome conjugation being rare.

In accordance with the foregoing scheme of classification all the haploid plants so far reported fall into the various groups as shown below:

I. MONOHAPLOIDS

1. *Datura* type (A)

<i>Datura stramonium</i>	(2n = 12)
<i>Lycopersicum esculentum</i>	(2n = 12)
<i>Crepis capillaris</i>	(2n = 3)
? <i>Crepis tectorum</i>	(2n = 4)
<i>Nicotiana glutinosa</i>	(2n = 12)
<i>Portulaca grandiflora</i>	(2n = 9)
<i>Zea mays</i>	(2n = 10)
<i>Pharbitis Nil</i>	(2n = 15)

2. *Oryza* type (A)

<i>Oryza sativa</i>	(2n = 12)
<i>Nicotiana Lungsdorffii</i>	(2n = 9)
<i>Nicotiana sylvestris</i>	(2n = 12)
<i>Triticum monococcum</i>	(2n = 7)

3. *Oenothera* type (A)

<i>Oenothera franciscana</i>	(2n = 7)
<i>Oenothera blandina</i>	(2n = 7)
<i>Oenothera Hookeri</i>	(2n = 7)
? <i>Oenothera rubricalyx</i>	(2n = 7)
? <i>Oenothera argillicola</i>	(2n = 7)

4. *Matthiola* type (A + frag.)

<i>Matthiola incana</i>	(2n = 7 + frag.)
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(*Gossypium Davidsonii* haploids - 2n = 13 -- belong to tetra monohaploids, but, due to the fact that their meiosis has not been studied, it is impossible to place them in a definite type group).

II. POLYHAPLOIDS

A. *Allohaploids*1. *Nicotiana* type (AB)

Nicotiana tabacum (2n = 24)

Nicotiana rustica (2n = 24)

Digitalis mertonensis (2n = 56)

?*Gossypium barbadense* (2n = 26)

?*Gossypium hirsutum* (2n = 26)

2. *Brassica* type (AB)

Brassica Napella (2n = 19)

(*Triticum dicoccum*, *Tr. persicum*, *Tr. durum*, and *Tr. turgidum* haploids for all 2n = 14 belong either to the *Nicotiana* or *Brassica* type, but it is impossible to place them definitely in one or the other group, since the meiosis of these haploids has not been studied).

3. *Solanum* type (AAB)

Solanum nigrum (2n = 36)

Nicotiana rustica-paniculata (2n = 36)

4. *Triticum* type (ABC)

Triticum vulgare (2n = 21)

Triticum compactum (2n = 21)

5. *Aegilotriticum* type (ABCD)

Aegilotriticum (2n = 28)

B. *Pseudohaploids* (AA)

Oenothera Lamarckiana gigas (2n = 14)

Oenothera biennis gigas (2n = 14)

The proposed classification cannot, of course, be considered final. It will, naturally, be subject to alteration as new haploid plants are obtained and more detailed studies are made of those already known. A few haploids, marked in the list with a query, have been placed in one or another group tentatively, since their meiosis was not studied in detail by the investigators who obtained and reported them.

SUMMARY

1. With the aim of producing haploids experimentally in *Nicotiana rustica* the following methods of stimulating egg-cells were tested: (1) Irritation of the ovarian tissues by puncturing them with a fine needle, (2) determination of the effect of pollen tubes growing within the style, by removing the style after varying periods (5-24 hours) subsequent to pollination, (3) pollination with pollen from plants belonging to other genera of the *Solanaceae*; and (4) pollination with X-rayed pollen. Of all the methods tested only the last-mentioned, i.e., pollination with X-rayed pollen, proved to be successful as regards obtaining haploids.

2. During the experiments on obtaining haploids detailed observations were made as to the effect of X-rays on the fertilizing capacity of the generative nuclei of X-rayed pollen. The principal conclusions were as follows:

- (1) Heavy X-ray doses (12,000-23,000 *r*) induce irregularities in the nuclear divisions of the pollen, as a result of which imperfect and, probably, inviable generative cells are formed.
- (2) The number of seeds per capsule begins to decrease at an X-ray dosage of 8,000 *r*, this dosage evidently being adequate to lower the fertilizing capacity of the generative cells.
- (3) With further increase of the X-ray dosage the set of seed rapidly decreases, dropping to a few seeds in occasional capsules when the dosage reaches 23,000 *r*.
- (4) Doses exceeding 23,000 *r* proved to be adequate to sterilize completely the pollen of *N. rustica* var. *texana*, while to sterilize the pollen of var. *humilis* the dosage had to be somewhat higher - 26,000-30,000 *r*.
- (5) The germinating capacity of the seeds and the ability to survive of the seedlings were, in general, very low (beginning with an X-ray dosage of 12,000 *r*) and markedly decreased with the further increase in the X-ray dosage given the pollen.

3. In all there were obtained four haploid *N. rustica* plants. One of these, of the variety *humilis*, was found among seventeen plants obtained from pollination with *texana* pollen irradiated with an X-ray dosage of 17,000 *r*. The other three haploids are of the variety *texana*, and were found among twenty-one plants obtained by the

use of X-ray doses ranging from 26,000 to 30,000 r. The haploids of both varieties, therefore, were obtained by using X-ray doses close to those completely sterilizing the pollen.

4. From flowers pollinated with pollen given X-ray doses of 15,000 r, 21,000 r, and 23,000 r there were produced seven diploid plants ($2n = 48$) of the maternal type, which arose as a result of apomixis induced by pollination with X-rayed pollen.

5. Morphologically the *N. rustica* haploids, both of the variety *humilis* and of the variety *texana*, resemble the maternal plants, differing from them in reduced vigor, smaller size of leaves and flowers, and greater sterility. Somatic cells in the meristem of the roots of the haploids are slightly over half, while their nuclei are exactly half, the size of those in diploids.

6. At meiosis of the pollen mother-cells of the *N. rustica* haploids the 24 univalent chromosomes are distributed at random to the poles, being scattered at metaphase of the first division throughout the spindle area and even outside its limits (as many as one-third of the PMC's had from one to nine univalents outside the spindle area). Non-reduction was of comparatively frequent occurrence. Very rarely pollen mother-cells were encountered having one "bivalent".

7. Sporad formation in the haploids is irregular. The sporads differ in size, number of cells, size of nuclei, and number of nuclei per cell. Monads, diads, triads, tetrads, pentads, etc. - up to eight-celled sporads - are formed. The diads and monads are, in the majority of cases, viable.

8. Megasporogenesis proceeds in practically the same manner as microsporogenesis. Most of the megaspores prove not to be viable and perish very early. The few embryo sacs formed develop normally and have the same structure as the embryo sacs of diploid plants.

9. From the few seed resulting from open pollination of the haploid plants the progeny was mostly diploid, with the exception of a few plants which proved to be polyploid. As yet the chromosome number of only one of the latter has been determined; it was found to be 72 (triploid).

10. As a result of pollination of the *N. rustica* haploids with pollen of other species several hybrids were obtained [*N. rustica* haploid ♀ × *N. paniculata* ♂ ($2n = 36$)], having at meiosis 12 bivalents and 12 univalents.

11. In the "Discussion" a survey is made of the origin and peculiarities of all the haploids known at present among the flowering plants. On the basis of this survey the writer classifies haploids according to their origin (character of the maternal species) and their meiotic irregularities.

12. The *N. rustica* haploids, having in their haploid chromosome set two genomes of two different species, are referred in the proposed classification to the *Nicotiana* type of allo-haploids, which is characterized by the univalent condition of all chromosomes at the heterotypic division.

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APPENDIX

APPENDIX

SUMMARY TABLE

Species	Mode of origin of haploids	Chief morphological characteristics	Chrom. No	Type of heterotypic division in F ₁ C's	Sterility of haploids	Progeny obtained from haploids	Investigators
1	2	3	4	5	6	7	8
<i>Brassica Napella</i>	Arose spontaneously in field cultures of <i>B. Napella</i> .	Smaller in all parts, as compared with diploids, size of leaves and flowers, especially reduced	19	From 19 to 5 ₁ plus from 0 to 7 _{II}	Very high degree of sterility	None	MORINAGA and FUKUSHIMA, 1933
<i>Crepis capillaris</i>	1 In F ₁ <i>C. capillaris</i> ♀ (2n = 6) × <i>C. tetorum</i> ♂ (2n = 8) 2 In F ₂ <i>C. capillaris</i> ♀ × <i>C. neglecta</i> ♂ (2n = 8) 3 In F ₁ <i>C. capillaris</i> ♀ × <i>C. setosa</i> ♂ (2n = 8)	Maternal-type plants, but all parts proportionally reduced. Shape of leaf and character of branching somewhat altered. In one case there arose a normally fertile diploid branch	3	3 _I At first division non-reduction and division of univalents at 1A, with separation of the halves to the two poles or with their inclusion in a single nucleus	Completely sterile plants, seeds obtained only from the diploid branch	No progeny from haploid shoots, progeny from seeds from diploid branch normally fertile and homozygous with respect to morphological characters	HOLLINGSHEAD, 1928, 1930, BARCOCK and NAVASHIN, 1930
<i>Crepis tetorum</i>	By pollinating X-radiated (4,600 r) ovaries with untreated (normal) pollen (Experimental androgenesis)	Greatly reduced in size, possessing all characters of the paternal plant (the paternal plant had characters recessive to those possessed by the mother plant)	4	Not studied	Completely sterile, one diploid branch with normal fertility	None	GERASSIMOVA, 1936
<i>Datura stramonium</i>	1 By treatment with low temperature at the time of fertilization 2 In F ₁ <i>D. stramonium</i> (2n = 24) × <i>D. ferox</i> (2n = 24)	Greatly reduced, maternal-type plants	12	12 _I Lagging chromosomes at 1A; cases reported of non-reduction with formation of two nuclei having 12 chromosomes each	88% sterile	Haploids propagated by cuttings, the seeds thus obtained gave numerous progeny, consisting mostly of 2n plants, a few 3n and 4n plants, trisomics and gene mutations.	BLAKESLEE, BELLING, FARNHAM and BERGNER, 1922, BELLING and BLAKESLEE, 1923, 1927, BLAKESLEE and BELLING, 1924, BLAKESLEE, MORRISON and AVERY, 1927
<i>Gossypium hirsutum</i>	1 In field cultures of the variety "Acadia Okra" 2. One plant of twin seedlings from a polypenthyron seed		26				HARLAND, 1936
<i>Gossypium barbadense</i>	1. Among twin seedlings from polypenthyron seeds. 2. Spontaneously in field cultures; one haploid to 3-4,000 diploid plants.	Reduced maternal-type plants.	26		Highly sterile plants. Seeds obtained by pollination with pollen from 2n plants	Hybrid obtained between haploid and the diploid species, <i>G. arborescens</i> (2n = 26)	HARLAND, 1936.

Species	Mode of origin of haploids	Chief morphological characteristics	Chrom. No.	Type of heterotypic division in PMC's	Sterility of haploids	Progeny obtained from haploids	Investigators
1	2	3	4	5	6	7	8
<i>Gossypium Davidsonii</i>	In F_1 <i>Gossypium Davidsonii</i> $\delta \times G. trilobum$ δ .	Almost normal in height and vigor, but leaves reduced in size and branches shorter.	13	Not studied	—	—	SKOVSTED, 1935.
<i>Lycopersicon esculentum</i>	In an F_2 population from an interracial cross (spontaneous origin)	Dwarf plants with greatly reduced leaves and flowers	12	12 $\frac{1}{2}$ Rarely non-reduction.	99% abortive pollen. A few seeds obtained only by open pollination or by use of pollen from a diploid	Plants grown from seed proved to be diploid and uniform by decapitation of the haploids completely homozygous-2n and 4n plants were obtained	LINDSTROM, 1929; LINDSTROM and KOOS, 1931. ILLUPHREY, 1934
<i>Matthiola incana</i>	1 Spontaneously in the F_1 from the cross "Snowflake" variant (2n+1 tr) \times normal form	Dwarf plant of the maternal type	7 + tr	7 $\frac{1}{2}$ + tr A-a rule, non-reduction (70% of cases), dichotomosomes divide and halves pass to opposite poles	Sterility comparatively great	—	LENLEV and FROST, 1928
<i>Nicotiana glauca</i>	1 Spontaneously in a pure line. 2 In the F_1 from a cross between X-ray aberrants	Plants greatly reduced in size as compared with diploids, color of corolla less intensive	12	12 $\frac{1}{2}$	Completely sterile, no seeds obtained by open pollination or by using pollen of 2n plants	—	GOODSPEED and AVERY, 1929. WEBBER, 1933
<i>Nicotiana Langsdorffii</i>	In the F_1 from a cross between an aberrant <i>N. tabacum</i> δ (2n=70-72) and <i>N. Langsdorffii</i> δ (2n=18) (androgenesis)	Greatly reduced plant of the paternal type (<i>N. Langsdorffii</i>)	9	9 $\frac{1}{2}$ Sometimes occasional "bivalents" occur	92% abortive pollen, a very few seeds obtained by use of pollen from a diploid.	—	KOSTOFF, 1929
<i>Nicotiana rustica</i>	1. By pollination with X-rayed pollen 2. In F_1 <i>N. rustica</i> $\delta \times N. tabacum$ δ .	Reduced maternal-type plants, often of the same height as diploids, shape of leaf altered in the <i>N. rustica</i> var <i>humilis</i> haploid.	24	24 $\frac{1}{2}$ Sometimes occasional "bivalents."	97% abortive pollen, a very few seeds obtained by open pollination and by use of pollen from 2n plants	Progeny mostly 2n plants, a few polyploid forms (only 3n plants precisely established)	KOSTOFF (preliminary communication), 1936, the author of the present paper
<i>Nicotiana sylvestris</i>	In the F_1 from the cross: hybrid <i>N. tabacum-sylvestris</i> $\delta \times N. sylvestris$ δ (androgenesis).	Dwarf plant, paternal type (<i>N. sylvestris</i>).	12	12 $\frac{1}{2}$ Sometimes occasional "bivalents".	Very high degree of sterility.	—	KOSTOFF, 1934

Species	Mode of origin of haploids	Chief morphological characteristics	Chrom. No.	Type of heterotype division in PMC's	Sterility of haploids	Progeny obtained from haploids	Investigators
1	2	3	4	5	6	7	8
<i>Nicotiana tabacum</i>	1 In the F ₁ from crosses of <i>N. tabacum</i> ♂ with the ff species, <i>N. sylvestris</i> , <i>N. inermis</i> , <i>N. glauca</i> , <i>N. longiflora</i> , and <i>N. glauca</i> (♂) 2 In F ₁ , <i>N. glauca</i> ♀ × <i>N. tabacum</i> ♂ (androgenesis) 3 Spontaneously, by self-pollination of <i>N. tabacum</i> 4 By pollination of <i>N. tabacum</i> with pollen from <i>N. glauca</i> and treatment with low (+3°) and high (+45°) temperature	Practically all plants greatly reduced replicas of the maternal parent, in case of parthenogenetic origin, or of the pollen parent, in case of androgenetic origin. In a few haploids reduction in size not noticeable.	24	24 Some investigators report the occurrence of "bivalents" (CHUPMAN and GOODEN, 1922; CLAUSEN and LAMPERT, 1929; KRISTOFF, 1930; KESDAL, 1930; MCCRAY, 1932; TERNOSKY, 1936; HACHATUROV, 1937; FOMOLOCHKO, 1937) In occasional PMC's, from 0 to 12 "bivalents."	99% abortive pollen. Most of the numerous <i>N. tabacum</i> haploids do not produce seeds, from a few there were obtained a very small no. of seeds by using pollen from 2n plants	Normal diploid plants	CLAUSEN and MAXS, 1924; CHUPMAN and GOODEN, 1927; KRISTOFF, 1929; CLAUSEN and LAMPERT, 1929; KRISTOFF, 1930; KESDAL, 1930; MCCRAY, 1932; TERNOSKY, 1936; HACHATUROV, 1937; FOMOLOCHKO, 1937
<i>Oenothera argillicola</i>	In F ₁ , <i>O. argillicola</i> ♀ × <i>O. biennis</i> ♂	Reduced maternal-type plant	7		Highly sterile		STOMPS, 1930
<i>Oenothera biennis</i>	In F ₁ , <i>O. biennis</i> ♀ × <i>O. nemoralis</i> ♂	Greatly reduced plant with very small flowers and leaves, all parts about half normal size	7	7 In 80% of cases 71, 1-2 II Occasionally there occur 1 III or 1 IV	About 66% abortive pollen, sterility great but every capsule had a few seeds, by open pollination about 1% of the seeds set in each capsule	Progeny from open pollination mostly diploid plants, occasionally a haploid (out of 15 plants 14 were diploids and 1 a haploid)	CATHERINE, 1932
<i>Oenothera fransiscana</i>	1 Spontaneously in a pure line 2 In F ₁ , <i>O. fransiscana</i> ♀ × <i>O. fransiscana-sulcata</i> ♂ 3 In F ₁ , <i>O. fransiscana</i> ♀ × <i>O. longiflora</i> ♂	Reduced maternal-type plants	7	7 Sometimes from 1 to 2 "bivalents", division of univalents at 1A occurs quite frequently.	Up to 90% abortive pollen, a few seeds obtained by self-pollination and by use of pollen from 2n plants	Most plants diploid, a few haploid, in addition, there occurred two trisoma mutants (2n + 13)	EMERSON, 1929; DAVIS and KULKARNI, 1930; STOMPS, 1930a, b, 1931; LEIVELD, 1932; ANDERSON, 1933; BLEIER, 1933
<i>Oenothera hookeri</i>	1 In F ₁ , <i>O. hookeri</i> ♀ × <i>O. longiflora</i> ♂ 2 In F ₁ , <i>O. hookeri</i> ♀ × <i>O. argillicola</i> ♂	Reduced maternal-type plants	7	7 Rare formation of 1-2 "bivalents"	Completely sterile, 97% abortive pollen		STOMPS, 1930; BLEIER, 1933
<i>Oenothera rubricalyx</i>	In F ₁ , <i>O. rubricalyx</i> ♀ × <i>O. eriantha</i> ♂	Dwarf plant of maternal type; shape of leaves altered	7	Not studied.	Completely sterile, almost all pollen abortive		GATES, 1929; GATES and GOODWIN, 1930

Species	Mode of origin of haploids	Chief morphological characteristics	Chrom. No.	Type of heterotypic division in PMC's	Sterility of haploids	Progeny obtained from haploids	Investigators
1	2	3	4	5	6	7	8
<i>Oryza sativa</i>	1 Spontaneously in commercial sowings (frequency of occurrence — 0.0023%) 2 In F_1 , F_2 , F_3 and F_4 populations from intervarietal (intraspecific) crosses 3 Among seedlings grown from polyembryonic seeds, occurring in rice in the ratio 1 : 1000 4 Among the progeny of a highly sterile mutant	Reduced maternal-type plants; spike and leaves particularly reduced. Often perithecarpe development of fruit. Among a large number of haploid plants (vegetatively reproduced) a number of diploid shoots were observed	12	12 ₁ Rarely 1:2 "bivalent" are formed.	Very high sterility. From 1212 haploids (vegetatively reproduced) 41 seeds obtained by self-pollination, i.e. 0.0022%; by pollination with pollen from 2n plants percentage of successful pollination increased to 1.06-2.4	All plants diploid and normally fertile	MORINAGA and FUKUSHIMA, 1931, 1932, 1934, NAKAMURA, 1933, KANAMURA, 1933, KATAYAMA, 1933, KATAYAMA, 1933 and KANANJAM, 1933
<i>Pharbitis Nil</i>	1 In the F_1 from a cross between a normal plant (?) and a perithecal chimera (?) having a large amount of abortive pollen 2 Spontaneously in a pure line and from crosses between varieties	Reduced maternal-type plants. Change in leaf shape and very marked change in shape of crown reported	15	15 ₁ Rare formation of "bivalents" — single instances in occasional PMC's	Almost completely sterile, vegetatively reproduced	Among haploid shoots of vegetatively reproduced plants gene mutations were revealed	U', 1930, 1932, KATAYAMA, 1935b
<i>Portulaca grandiflora</i>	In the F_1 from an intraspecific cross	Plants only half the size of diploids	9	9 ₁	By self-pollination and pollination with pollen from 2n plants no seeds obtained, 5 seeds from pollination of 2n plants with pollen from the haploid	Progeny: all diploid and adapted to initial mother plants	OKURA, 1933
<i>Solanum nigrum</i>	1 By self-pollination of a perithecal chimera, <i>Solanum nigrum</i> (epidermis from <i>S. sisymbriifolium</i>) 2 In F_1 , <i>S. nigrum</i> \times <i>S. tuberosum</i>	Reduced maternal-type plants, change in the character of serration of leaves and compactness of inflorescence	36	12 ₁ 12 ₁ In general the number of bivalents varies from 3 to 12, with a corresponding number of univalent chromosomes.	Completely sterile; the 6 seeds obtained from the haploids, the investigator considers to be of apomictic origin.	From 6 seeds 3 plants grown having diploid chrom. no. Numerous progeny of these plants entirely homozygous	JORGENSEN, 1928
<i>Triticum monococcum</i>	1 Spontaneously in a pure culture (frequency of occurrence — 0.48%) 2 By pollination with X-rayed pollen (frequency of occurrence — up to 17.58%)	Reduced maternal-type plants	7	7 ₁ At diakinesis there was observed a ring of chromosomes; at IM rarely 1 "bivalent" (in up to 2% of PMC's).	Abortive pollen — up to 99.5%, vegetatively reproduced. From 8,285 spikelets (289 spikes) only 20 seeds obtained.	Of 6 plants grown from seed 3 were diploids and 3 haploids.	KIYAKA and KATAYAMA, 1932, 1933, CHIZAKI, 1933, KATAYAMA, 1934, 1935a.

Species	Mode of origin of haploids	Chief morphological characteristics	Chrom. No.	Type of heterotypic division in PMC's	Sterility of haploids	Progeny obtained from haploids	Investigators
1	2	3	4	5	6	7	8
<i>Triticum dicoccum</i>	By pollination with X-rayed pollen (2,200-2,500 r)	Reduced plant with new characters (shape of spike and pubescence of straw nodes) like those of <i>Tr. monacorum</i>	14	Not described	(completely sterile)	—	YEFERIN and VASILYEV, 1936
<i>Triticum persicum</i>	By pollination with X-rayed pollen (2,200-2,500 r)	Reduced plant, new characters (shape of spike and pubescence of nodes) made the haploid closely resemble <i>Tr. monacorum</i>	14	Not described	Completely sterile	—	YEFERIN and VASILYEV, 1936
<i>Triticum durum</i>	In F_3 , <i>Tr. durum</i> φ \times <i>Tr. monococcum</i> δ	Height of plants same as that of diploids; some characters of <i>Tr. monococcum</i> (general habit and pubescence of straw nodes)	14	Not described	(completely sterile)	—	VASILYEV, 1936
<i>Triticum turgidum</i>	By pollination of <i>Tr. turgidum</i> with pollen of <i>Secale cereale</i>	Reduced maternal-type plants	14	Not studied	Sterile	—	NAKAJIMA, 1935
<i>Triticum compactum</i>	In F_3 , <i>Tr. compactum humboldtii</i> φ \times <i>Aegilops cylindrica</i> δ	Haploid plant identical to diploid <i>Tr. compactum humboldtii</i> , differing only by its sterility	21	21 _I Very rarely from 1-2 "bivalents"	Flowers 99.8% sterile, 9 seeds obtained	—	GAINES and AASE, 1926
<i>Triticum vulgare</i>	1 In F_3 , F_4 and F_5 of interracial crosses 2 Among seedlings from polyploid embryonic seeds (2%)	Haploids characterized by later flowering, small spikes, and reduced number of spikelets per spike	21	21 _I in 47.7% of PMC's, from 1 to 4 _I in 52.3% of them. Also 1 _{III} in 0.93% of PMC's	Almost completely sterile plants, 98% sterility	—	YAMASAKI, 1934, 1936; NAKAKAWA and KAWAKAMI, 1934
<i>Zea mays</i>	1. By pollination with X-rayed pollen 2 Spontaneously in a pure culture.	Dwarf maternal-type plants	10	10 _I	Sterile	—	STADLER, 1931; KANDOLPH, 1932

ÜBER DIE PSEUDOGAME FORTPFLANZUNG BEI POTENTILLA

von

G. GENTSCHEFF

Laboratorium für Vererbungsforschung der Universität, Sofia

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Mit 1 Tafel.

Die Gattung *Potentilla* enthält eine Anzahl apomiktisch sich fortpflanzender Arten. Während MUNTZING (1928) die Fortpflanzungsverhältnisse der Gattung *Potentilla* auf experimentalem Wege untersucht hatte, machte POPOFF (1935) den Versuch, gleichzeitig experimentell und zytologisch diese Erscheinungen zu erforschen. Diesen Studien zufolge erscheint die Gattung *Potentilla* als ein geeignetes Objekt für die experimentelle und gleichzeitig zytologische Untersuchung des Problems. Wenn man in Betracht zieht, dass bei der Gattung *Potentilla* die Artkreuzungen technisch leichter durchzuführen sind als bei den Gattungen *Taraxacum*, *Hieracium* u.a., wo ebenso die obligat apomiktische Fortpflanzung eine oft vorkommende Erscheinung ist, so erhellt daraus, dass die Gattung *Potentilla* sich für eine Prüfung der Anschauungen eignet, wonach als Ursache für die ungeschlechtliche Fortpflanzung die Hybridisation betrachtet wird. (ERNST 1918).

Während der Jahre 1934, '35, '36 sind zum Zwecke einer solchen Prüfung an dem genetischen Laboratorium in Sofia zahlreiche Kreuzungen zwischen verschiedenen Arten der Gattung *Potentilla* vorgenommen worden. Die vorliegende Mitteilung bezieht sich nur auf eine vergleichend embryologische Untersuchung der Arten *P. nepalensis* ($2n=42$) und *P. argyrophylla* ($2n=63$), sowie des Bastards *P. nepalensis* ($2n=42$) \times *P. splendens* ($2n=28$).

Zum Zwecke der zytologischen Untersuchungen wurden Präparate

von Blütenknospen hergestellt. Während für das Studium der Reduktionsteilung in den Pollenmutterzellen das Material auf 8 Mikronen geschnitten wurde, wurde dasjenige für die embryologischen Untersuchungen auf 8–25 Mikronen geschnitten. Als Fixativ ist die NAVASCHIN'sche Lösung und als Färbemittel Haematoxylin Haidenhain verwendet worden.

Bei den Kreuzungsversuchen, in welchen *P. nepalensis* von 15 anderen *Potentilla*-Arten bestäubt wurde, erhielt man nur bei zwei Kreuzungen Bastarde und von keiner Kreuzung eine matroklone Nachkommenschaft, was uns zu der Überzeugung führt, dass *P. nepalensis* eine sexuell sich fortpflanzende Art ist.

Die andere von uns untersuchte Art *P. argyrophylla* hatte $2n = 63$ Chromosomen und war im Habitus eine echte *P. argyrophylla*, mit dem Unterschied, dass sie anstatt rot, wie die meisten *Argyrophyllen*, gelb blühte. Im Jahre 1935, in dem POPOFF sich mit ihr befasste, hatte die Pflanze keine aktiven Pollen. Die Beobachtungen im folgenden Jahre jedoch zeigten, dass diese Pflanze gleich ihrer Nachkommenschaft aktive Pollen produzierte. Deswegen sind wir geneigt, die von POPOFF beobachtete Pollensterilität auf physiologische Störungen zurückzuführen.

Von dem Bastard *P. nepalensis* × *P. splendens* wurden nur zwei gut entwickelte und reichlich blühende Pflanzen gezogen, welche ihrem Aussehen nach ein Mittelding zwischen den Eltern waren. Beide Hybriden waren vollständig steril. Die zytologischen Untersuchungen haben bewiesen, dass die Pollenmutterzellen schon in der Synapsis degenerieren.

Aus den erwähnten Kreuzungsversuchen erhellt, dass die eine Elternpflanze: *P. nepalensis* sich geschlechtlich fortpflanzt; in Bezug auf die andere Elternpflanze jedoch haben wir keine Angaben, da dieselbe sofort nach vollzogener Hybridisation in Verlust geraten war. Aus den Angaben von POPOFF (1935, S. 511, Tabelle I) ist aber ersichtlich, dass diese *P. splendens* Pflanze bestäubt mit Pollen von vierzehn anderen *Potentilla*-Arten in keinem Fall matroklone Nachkommenschaft hervorbrachte, während sie als Bestäuber von drei anderen Arten eine solche Nachkommenschaft stimulierte. Dies lässt uns vermuten, dass die Pflanze zu einer sich geschlechtlich fortpflanzenden Art gehört.

Die Embryoentwicklung der Gattung *Potentilla* ist von JÖNSSON

(1879/80), VESQUE (1879) und PECHOUTRE (1902) untersucht worden. Verhältnismässig jünger sind die Untersuchungen von FORENBACHER (1913). Neuestens hat POPOFF (1935) auf Grund experimenteller und zytologischer Angaben zur Aufklärung der bei dieser Gattung vorkommenden Pseudogamie beigetragen. Durch diese Untersuchungen ist er zu dem Resultat gelangt, dass bei einigen Fällen Aposporie, bei anderen Nucellarembryonie durch das Wachstum der eigenen wie der fremden Pollen hervorgerufen wurde.

Bei unseren Untersuchungen wurden 50 Blüten der Hybride *P. nepalensis* × *P. splendens* mit Pollen von *P. nepalensis* bestäubt. Ebensoviel Blüten wurden isoliert und blieben unbestäubt. Dabei wurde keine Kastrierung vorgenommen, da wie erwähnt, die Pollenmutterzellen dieser Hybride schon in der Synapsis degenerieren. Die für die zytologischen Untersuchungen bestimmten bestäubten und unbestäubten Blüten wurden in der Folge zwölf Tage hindurch je nach 24 Stunden fixiert. Ausserdem wurden verschieden grosse Blütenknospen fixiert, um eine Untersuchung der Stadien vor der Embryoentwicklung zu ermöglichen. Auf die gleiche Weise wurde die Embryoentwicklung bei *P. nepalensis* und *P. argyrophylla* untersucht.

Es ist bekannt, dass viele Gattungen der Rosaceen, zu welchen auch *Potentilla* gehört, mehrzelligen weiblichen Archespor besitzen (SCHNARF 1931). Mit fortschreitender Entwicklung differenzieren sich die Archesporzellen; nur diejenige, die eine Mittelstellung hatte, vergrössert ihren Kernumfang und tritt in ein synapsisähnliches Stadium ein (Tafel I, Abb. 1). Sehr oft erscheinen gleichzeitig 2 oder 3 solcher Zellen, wobei sie nach deren Degeneration durch andere, ihnen gleiche lateral oder unterhalb angeordnete ersetzt werden (Tafel I, Abb. 2). Gewöhnlich geht eine von den Embryosackmutterzellen der Blüten, die sich in einem Stadium unmittelbar vor dem Blühen befinden, eine Reduktionsteilung ein. Mit fortschreitender Entwicklung bildet eine solche Embryosackmutterzelle den Embryosack, der sich dann in der Länge der Samenanlage erweitert (Tafel I, Abb. 3). Es ist zu bemerken, dass bei *P. nepalensis* eine Reduktionsteilung in den Embryosackmutterzellen selten beobachtet wurde. Unter einigen Hunderten Samenanlagen konnten wir nur in zwei Fällen Embryosackmutterzellen in der Diakinese beobachten, wo 21 bivalente Chromosomen sehr deutlich zu unterscheiden waren. Einen

solchen Fall zeigt uns Abbildung 1a und 1b, wo die Chromosomen in zwei Schnitten zu sehen sind. In keinem Fall wurde Tetradenbildung beobachtet, was uns in dem Glauben bekräftigt, dass sich in diesem

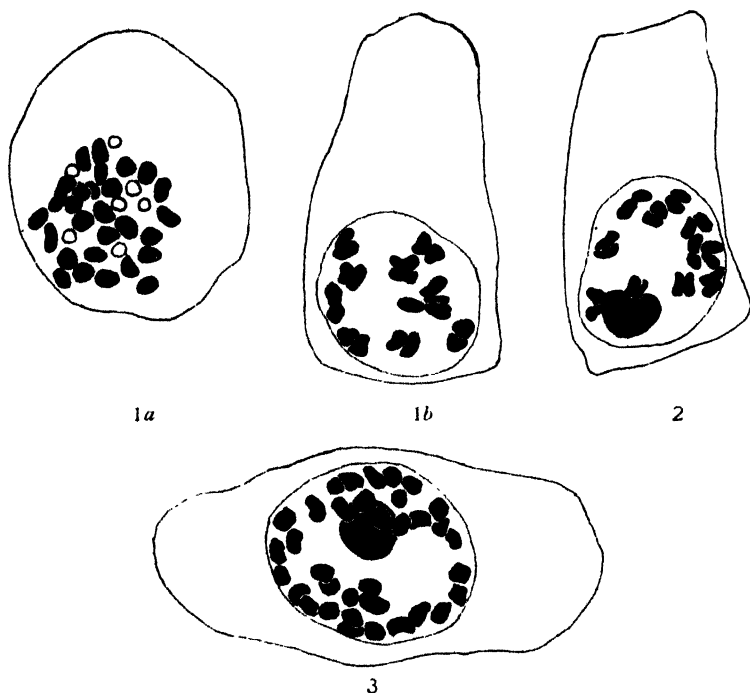


ABB 1-3 *P. nepalensis* Embryosackmutterzelle in Diakinese (1a, b), *P. argyrophylla* Pollenmutterzelle in heterotyper Metaphase (2), *P. nepalensis* × *P. splendens* Embryosackmutterzelle in Diakinese (3)
Vergross. etwa 1850 mal

Falle der Embryosack direkt aus der Embryosackmutterzelle entwickelt.

Bei kastrierten Blüten von *P. nepalensis*, beginnt die Degeneration der Embryosäcke erst 4-5 Tage nach dem Verblühen, wogegen bei selbstbestäubten Blüten 3 Tage nach der Bestäubung 2-5 zellige Embryos in einer verhältnismässig geräumigen Embryosackhöhle zu sehen sind (Tafel I, Abb. 4).

Bei *P. argyrophylla* ($2n=63$) verläuft die Embryoentwicklung bei den selbstbestäubten Blüten auf gleiche Weise wie bei den mit frem-

den Pollen bestäubten. Nach Angaben von POPOFF (1935) hatte die Pflanze $2n = 63$ Chromosomen und zeichnete sich durch eine pseudogame Fortpflanzung aus. Unsere zytologischen Untersuchungen der Pollenmutterzellen ergaben, dass während der heterotypischen Metaphase 28 bivalente und 7 univalente Chromosomen zu sehen waren (Abb. 2), was zu der Vermutung Anlass gibt, dass diese Pflanze eine hybride Herkunft habe. Ausserdem sind wir der Ansicht, dass die grosse Zahl bivalenter Chromosomen einen verhältnismässig grossen Prozentsatz wachstumfähiger Pollen bedingt. Dass diese Pflanze



ABB. 4a-e Kronenblätter von *P. argyrophylla* ($2n = 56$) (a), *P. argyrophylla* ($2n = 63$) (e) und von drei F_1 Hybriden der Kreuzung *P. argyrophylla* ($2n = 56$) \times *P. argyrophylla* ($2n = 63$) (b, c, d).

einen wachstumfähigen Pollen besitzt, zeigen jene Versuche, bei welchen *P. argyrophylla* ($2n = 63$) mit 11 anderen Arten der Gattung *Potentilla* (*P. sanguisorbifolia*, *P. Knapii*, *P. aurca*, *P. nepalensis*, *P. Hookeriana*, *P. atrosanguinea*, *P. procumbens*, *P. Gibsonis*, *P. argyrophylla* ($2n = 56$), *P. norvegica* und *P. Kippiana*) gekreuzt wurde. So wurde bei den Kreuzungen, bei welchen *P. argyrophylla* ($2n = 63$) als Mutterpflanze verwendet wurde, matroklinaler Nachkommenschaft erhalten, (*P. argyrophylla* ($2n = 63$) \times *P. nepalensis* ($2n = 42$), *P. argyrophylla* ($2n = 63$) \times *P. atrosanguinea* ($2n = 56$), *P. argyrophylla* ($2n = 63$) \times *P. Gibsonis* ($2n = 56$) und *P. argyrophylla* ($2n = 63$) \times *P. argyrophylla* ($2n = 56$)), wogegen aber die reziproken Kreuzungen nur echte Hybriden hervorbrachten (*P. argyrophylla* ($2n = 56$) \times *P. argyrophylla* ($2n = 63$) und *P. norvegica* ($2n = 70$) \times *P. argyrophylla* ($2n = 63$)). Der Bastardcharakter der Pflanzen dieser Kreuzungen ist an dem gesamten Habitus, besonders an Grösse, Form und Farbe der Kronenblätter zu erkennen. In Bezug auf die Kronenblätter gibt Abbildung 4 Aufschluss über die Aufspaltung bei der Kreuzung *P. argyrophylla* ($2n = 56$) \times *P. argyrophylla* ($2n = 63$). In der Abbildung ist mit a das rote Kronenblatt von *P. argyrophylla* ($2n = 56$), mit e das

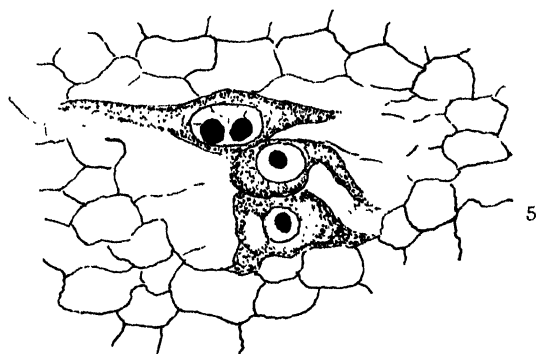
gelbe Kronenblatt von *P. argyrophylla* ($2n=63$) und mit *b*, *c*, *d* sind die Kronenblätter von den 3 hybriden Pflanzen bezeichnet. Die zytologischen und experimentellen Beobachtungen über die Mutterpflanzen (*P. argyrophylla* ($2n=56$) und *P. norvegica* ($2n=70$)), die an den beiden erwähnten Kreuzungen teilnahmen, zeigen, dass dieselben durch eine normale Meiosis und sexuelle Fortpflanzung gekennzeichnet sind. Dies beweist andererseits, dass in den erwähnten Kreuzungen die beobachtete Aufspaltung durch die Vaterpflanze *P. argyrophylla* ($2n=63$) bedingt ist.

Unsere Untersuchungen stellen fest, dass die anfänglichen Stadien in der Embryosackentwicklung von *P. argyrophylla* sich nicht von jener der *P. nepalensis* unterscheiden. Auffallende Abweichungen treten erst in späteren Stadien ein. Bei *P. argyrophylla* sind im Gegensatz zu *P. nepalensis* selten unmittelbar vor dem Blühen fertige Embryosäcke beobachtet worden, wogegen jedoch nach der Bestäubung fast in jeder Samenanlage Anzeichen von Embryosackbildungen erkennbar waren. Samenanlagen mit mehreren Embryosäcken sind häufig beobachtet worden. So zeigt Abbildung 5 (Tafel I) den mittleren Teil einer Samenanlage von solchen Blüten, die einen Tag nach der Bestäubung fixiert wurden und in welchen deutlich 3 Embryosäcke zu sehen sind.

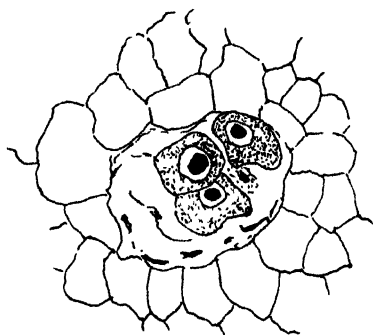
Die verhältnismässig sehr geringe Zahl von Embryosäcken in den Blüten, die kurz vor ihrem Öffnen fixiert worden sind, zeigt, dass bei einer grossen Zahl von Samenanlagen, die Embryosackmutterzellen meist in einem Stadium vor der Embryosackentwicklung degenerieren. Andererseits beweist das Erscheinen einer grossen Zahl von Embryosäcken nach der Bestäubung, dass dieselben eine durch das Pollenwachstum stimulierte apomiktische Herkunft haben.

SCHIMOTOMAI (1935) ist der Ansicht, dass der Ausfall der Reduktionsteilung in der Embryosackmutterzelle von *P. hirta* die Bildung von diploiden Embryosäcken bedingt. Bei *P. argyrophylla* ($2n=63$); sind wir nicht geneigt, eine solche Entwicklung zu vermuten, da wir nicht imstande waren, solch einen Vorgang in den Embryosackmutterzellen zu beobachten. Auf Grund oben auseinandergesetzter Erfahrung sind wir zu vermuten geneigt, dass *P. argyrophylla* eine obligat apomiktische Art sei, deren Fortpflanzung einem durch Pollenwachstum stimulierten aposporen Vorgang zuzuschreiben ist.

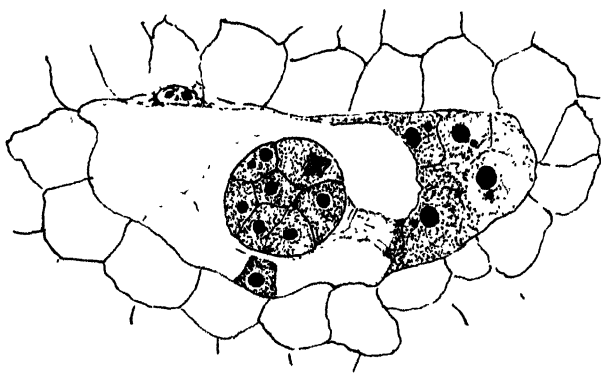
Gewöhnlich beginnt die Embryobildung bei *P. argyrophylla* 3 Tage



5



6



7

ABB 5 7. *P. nepalensis* \times *P. splendens*. Embryonal sich entwickelnde Zellen in den Nucellus (5, 6), Embryo, fünf Tage nach der Bestäubung (7) Vergröss. etwa 500 mal.

nach der Bestäubung (Tafel I, Abb. 6). Es ist andererseits zu bemerken, dass trotz zahlreicher Embryosäcke, die in einer Samenanlage in Erscheinung treten, sich immer nur ein Embryo bildet, u. zw. in jenem Embryosack, dessen Lage und Alter seine raschere Entwicklung begünstigt.

Anders vollzieht sich der Vorgang in der Embryoentwicklung bei der Hybride *P. nepalensis* \times *P. splendens*. Es ist zu bemerken, dass diese Hybride allein keine Samen ansetzt, jedoch mit Pollen von *P. nepalensis* bestäubt, Samenansatz aufweist. Aus diesem Grunde wurden zytologische Untersuchungen über die Embryoentwicklung in noch nicht geöffneten Blütenknospen, mit unbestäubten und vorher isolierten Blüten und in mit Pollen von *P. nepalensis* bestäubten Blüten vorgenommen. Die Resultate haben bewiesen, dass die Embryoentwicklung nach dem Verblühen bei den unbestäubten anders als bei den bestäubten verläuft.

Die ersten Stadien in der Embryosackentwicklung dieser Hybride sind mit denjenigen von *P. nepalensis* identisch. Während bei *P. nepalensis* die Samenanlagen von Blüten, die unmittelbar vor dem Öffnen fixiert wurden, gut entwickelte Embryosäcke enthalten, konnten wir bei *P. nepalensis* \times *P. splendens* trotz der grossen Zahl von Samenanlagen, die wir untersuchten, keinen Embryosack finden. Es hat sich herausgestellt, dass in den Samenanlagen der Blüten, die 1–6 Tage nach dem Öffnen fixiert wurden, keine Spur von Embryosäcken vorhanden war. Diese frühzeitige Degeneration in den Embryosackmutterzellen entspricht der in den Pollenmutterzellen beobachteten Degeneration während der Synapsis. Nur einem einzigen Fall begegneten wir, wo die Embryosackmutterzelle sich scheinbar in Diakinese vorfand und zwar hatte es den Anschein, als sei eine grosse Anzahl von univalenten Chromosomen vorhanden (Abb. 3).

Wie schon erwähnt, verläuft die Embryoentwicklung dieser Hybride während des Pollenwachstums auf gänzlich verschiedene Art. So ist bei Blüten, die mit Pollen von *P. nepalensis* bestäubt und sukzessive 1–12 Tage nach der Bestäubung konserviert wurden, eine gewisse Zahl von Samenanlagen (10–20%) in embryonaler Entwicklung beobachtet worden. Die Textabbildungen 5 und 6 (Abb. 7, Tafel I) stellen den Inhalt von Samenanlagen, die 2 Tage nach der Bestäubung fixiert wurden, dar. In diesen Samenanlagen sind unter den gewöhnlichen Nucellarzellen 3 verhältnismässig grössere, plasma-

reiche vorhanden, die in jenem Teil der Samenanlage eingefügt sind, in dem sich gewöhnlich der Embryosack entwickelt. Die in späteren Stadien gemachten Beobachtungen veranlassen uns zu vermuten, dass bei den weiteren Teilungen die Zellen ein embryonales Gewebe bilden, weshalb erst am fünften Tag nach der Bestäubung ein gut entwickelter aber in eine schmalere Embryosackhöhle eingebetteter Embryo zu sehen ist (Textabb. 7; Abb. 8, Tafel I).

Die erwähnten Beobachtungen über die Embryoentwicklung beweisen, dass das Pollenwachstum unerlässlich für die embryonale Entwicklung gewisser somatischer Zellen der Samenanlage ist. Aus ihrer Lage lässt sich der Schluss ziehen, dass diese Zellen sich in denjenigen Teilen der Samenanlage befinden, in welchen bei geschlechtlicher Fortpflanzung die Eizellen und Synergiden zu finden sind. Aus den gemachten Erfahrungen geht jedoch nicht klar hervor, wie aus den embryonal sich verhaltenden Nucellarzellen der Embryo entsteht. Obwohl selten, wurde doch als Folge des Pollenwachstums eine Aneinanderreihung von Zellen beobachtet, die sehr an eine einem Embryosack ähnliche Höhle erinnerte. Manchmal ist sogar in einer solchen ein Embryo beobachtet worden, der sich in jenem Teil der Höhle befindet, die bei einem normalen Embryosack der Stelle der Eizelle entspricht. (Abb. 9, Tafel I). Dies führt uns zu der Überzeugung, dass in solchen Fällen die Embryoentwicklung doch einen aposporen Ursprung habe.

Die Ansichten bezüglich der Ursachen, welche die embryonale Entwicklung einer unbefruchteten Eizelle oder einer gewöhnlichen somatischen Zelle bedingen, sind verschieden. HABERLANDT (1921–1927) hat einen Versuch unternommen, die Vorgänge aufzuklären. Auf Grund seiner Untersuchungen, die Einflüsse betreffend, welche die Nekrohormone auf die Zellteilung ausüben, gelangte er zu dem Resultat, dass sie von Bedeutung für die apomomiktische Fortpflanzung seien. So ist er der Ansicht, dass die Entartung der Synergiden, sowie der Nucellarzellen in irgendwelcher Beziehung zur parthenogenetischen Fortpflanzung stehe, welche bei *Hieracium* und *Taraxacum* beobachtet wurde. Er ist geneigt, ebenso die apospore Entwicklung in der Untergattung *Pilosella* denselben Ursachen zuzuschreiben. Andererseits nimmt er seinen Erfolg, durch Verletzungen in den unbefruchteten Samenanlagen von *Oenothera*, *Lamarckiana* adventive Embryonen zu erzeugen, als Bestätigung seiner Vermutungen an.



Abb. 1. *P. nepalensis*. Mehrzelliges weibliches Archespor; eine von den Embryosackmutterzellen in Synapsis



Abb. 2. *P. nepalensis*. Mehrzelliges weibliches Archespor mit Resten von degenerierten Embryosackmutterzellen

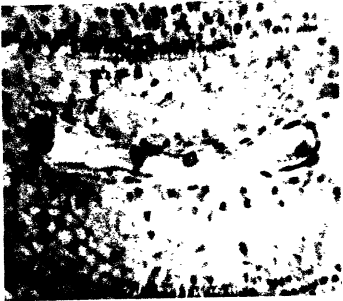


Abb. 3. *P. nepalensis*. Vollausgebildeter Embryosack vor Verschmelzung der Polkerne

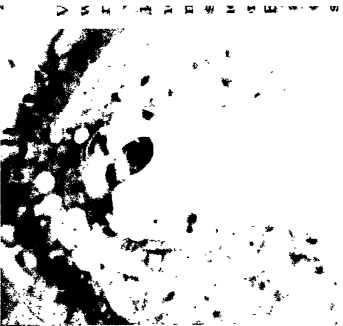


Abb. 4. *P. nepalensis*. Embryo, drei Tage nach der Bestäubung



Abb. 6. *P. argyrophylla*. Embryo, drei Tage nach der Bestäubung

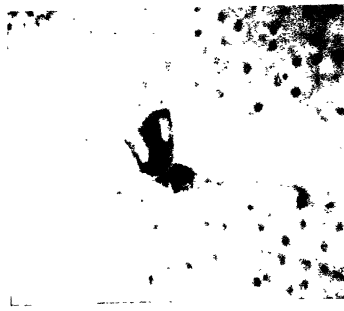


Abb. 7. *P. nepalensis* \times *P. splendens*. Embryonal sich entwickelnde Zellen in der Nuzellus (Textabb 5)



Abb. 8. *P. nepalensis* \times *P. splendens*. Embryo, fünf Tage nach der Bestäubung (Textabb 7).

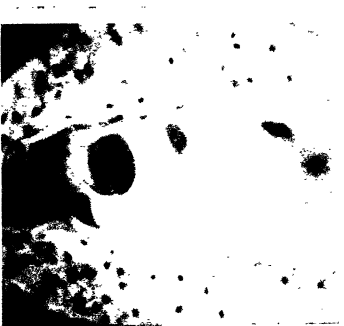


Abb. 9. *P. nepalensis* \times *P. splendens*. Embryo, zwei Tage nach der Bestäubung.

Unserer Ansicht nach könnten die Erscheinungen, welche die pseudogame Fortpflanzung bei *Potentilla* begleiten, durch die Voraussetzungen von HABERLANDT, nicht vollständig aufgeklärt werden. Unseren Beobachtungen zufolge waren bei den *Potentilla*-Arten in den allerersten Stadien der Embryoentwicklung eine Reihe von Degenerationserscheinungen wahrnehmbar, welche ebenso charakteristisch für die sexuell, wie für die apomiktisch sich fortpflanzenden Arten sind. Als Folge dieser Vorgänge ist der mittlere Teil der Samenanlage u. zw., dort, wo sich der Embryo ausbilden muss, mit Resten von degenerierten Embryosackmutterzellen ausgefüllt. Die Degenerationsprodukte allein sind hier nicht für die nachträgliche embryonale Entwicklung massgebend, denn wie schon erwähnt, tritt diese Entwicklung erst mit Beginn des Pollenwachstums ein. Es ist nicht ausgeschlossen, dass einerseits das Vorhandensein solcher Degenerationsprodukte, bezw. die dadurch gebildeten Nekrohormone, andererseits die stimulative Einwirkung des Pollenwachstums die pseudogame Entwicklung bedingen.

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ERRATA

Genetica XX, afl. 3 en 4, Art. G. FALKENSTRÖM:
Some animal species-crossings in nature with analyses
of similar ones in cultures, together with some funda-
mental questions discussed:

Page 233 on the 6th row from below read ontogene-
tical,

„ 239 in the head of Table 1 for "in de" read n. de,

„ 240 on the 9th row from below for "In" read I,

„ 244 in Table 2 on the 5th row (5e) under ms2
for "n. de" read n. la,

„ 271 on the 9th row from below read excused,

„ 280 on the 17th row read Carolyn Hörner,

In the references of the text to Plate I, II and III
read Plate III, IV and V respectively.

VERSUCH DER EINWIRKUNG VON X-STRAHLEN AUF *APOCYNUM VENETUM*

(Aus den Arbeiten der Tschui-Station des Instituts für Neuen Rohbast)

von

E. SAUROW

(Manuskript eingegangen am 10. Juli 1937)

Die zahlreiche Literatur über die Einwirkung der X-Strahlen auf lebende Organismen zerfällt in zwei Teile: 1) die Erzeugung von Mutationen und 2) die physiologische Einwirkung der Strahlen auf das Wachstum und die Entwicklung des Organismus.

Aus Mangel an Raum umgehen wir in unserem Artikel den ersten Teil sowie die Arbeiten, die mit der Einwirkung der X-Strahlen auf Tiere in Zusammenhang stehen. Von den zahlreichen Versuchen mit Pflanzen erwähnen wir hier bloss die Arbeiten, die mit unseren Experimenten in näherer Beziehung stehen.

So weisen die Forscher, A. W. und L. M. KOLTZOW (6) auf Grund ihrer Versuche mit Erbsen (*Pisum sativum*), die aus Samen herangezogen wurden, welche einer Bestrahlung mit X-Strahlen in trockenem Zustande unterzogen wurden, darauf hin, dass zu Beginn des Versuches die Pflanzen sich gleichmässig entwickelten und erst nach einiger Zeit vom Moment des Aufgangs der Pflanzen, die mit kleinen Dosen bestrahlten Pflanzen sich intensiver entwickelten. Dabei betonen sie die stimulierende Wirkung der grossen Dosen auf die Beschleunigung der Entwicklungsphasen der Erbse.

Die von denselben Verfassern durchgeführten Versuche der Röntgenisierung trockener und aufgegangener Weizensamen haben gezeigt, dass die bestrahlten, gekeimten Samen stärker beeinflusst waren, da sie eine Veränderung der Entwicklungsphasen und stimulierenden Effekt hervorriefen.

TSSCHECHOW (10) kommt auf Grund zahlreicher Experimente zu dem Schlusse, dass die X-Strahlen eine physiologische Einwirkung nicht nur auf aufgekeimte Samen und erwachsene Pflanzen ausüben, sondern auch auf trockene Samen.

ZURJUPA (9) weist darauf hin, dass bestimmte Dosierungen der X-Strahlen auf die Baumwollstaude eine positive Wirkung ausübten. Die Fristen des Eintretens einzelner Entwicklungsphasen wurden gekürzt, das Wachstum und der Ertrag nahmen zu. Dabei vermerkt er eine verschiedenartige Empfindlichkeit verschiedener Pflanzenarten zu den X-Strahlen.

DOROSCHENKO (5) bestätigt gleichfalls die Schlüsse über die beträchtliche Differenz einer Reihe von Kulturen in bezug auf ihre Empfindlichkeit den X-Strahlen gegenüber. Eine Röntgenisierung bei geringem Strom zeigte auf das Wachstum und die Entwicklung des Hafers und der Hirse eine positive Wirkung

TUSCHNJAKOWA und WASSILJEWSKI (8) liefern Data über die Einwirkung bestimmter R-Dosen auf die Abbreviation der Entwicklungsphasen bei der Tschufa (*Cyperus esculentus*), Soja und *Lupinus*. Bei der Tschufa wurde unter der Einwirkung der X-Strahlen intensiveres Wachstumsvermögen und grössere Produktivität erhalten; bei der Melone — Zunahme der Heranreifungsenergie.

ATABEKOWA (1) bestätigt durch ihre Experimente mit der Erbse die Ergebnisse, dass die Einwirkung der R-Strahlen auf Pflanzen in völliger Abhängigkeit von der physiologischen Aktivität der Samen steht. Trockene Samen herabgesetzter Lebenstätigkeit erscheinen äusseren Einwirkungen gegenüber resistenter, deswegen muss man bei ihnen zur Erhaltung einer Stimulation unbedingt grosse Dosen anwenden. Für eingeweichte Samen dagegen oder für Keimlinge genügen geringere Dosen der X-Strahlen, um einen physiologischen Effekt hervorzurufen.

BRESLAWETZ und AFANASSJEW (4), BRESLAWETZ und ATABEKOWA (3) kommen zu dem Schlusse, dass kleine Dosen der X-Strahlen das Wachstum und den Ertrag der Erbse und des Roggen stimulieren, grosse Dosen dagegen dieselben herabsetzen.

Unser Ziel war die Möglichkeit einer Stimulierung des Wachstums und der Entwicklung bei *Apocynum venetum* L. in den Bedingungen des Tschui-Tales (Zentralasien) festzustellen. Zu diesem Zwecke

wählten wir eine Amu-Darji'sche Form des *Apocynum venetum* ¹⁾. Letzterer ist eine perennierende Pflanze. Im ersten Vegetationsjahr hat er eine geringe Anzahl späterer Blüten, die gar keine Samen erzeugen; im zweiten Jahr setzen sie Keimblätter an, jedoch in einem die Selektionsarbeit durchaus nicht befriedigenden Quantum. Und nur im dritten Jahr erhalten wir die nötige Anzahl Samen.

Auf diesen Angaben fussend stellten wir uns die Aufgabe den Entwicklungszyklus des *Apocynum venetum* zu beschleunigen um im ersten oder im Notfall im zweiten Lebensjahr die für die Selektion benötigten Samen zu erhalten.

DIE BEDINGUNGEN DES EXPERIMENTS

Der Einwirkung der X-Strahlen unterlagen trockne Samen des Amu-Darji'schen *Apocynum* der Ernte von 1931 im Wissenschaftlichen Forschungsinstitut für Röntgenologie und Radiologie in Moskau. Bei der Bestrahlung der Samen mit X-Strahlen wurde der Verdreifachungsapparat ausgenützt und zwar bei folgenden Bedingungen: 180 kw 4 m A. Filter 0.5 Al, Abstand 20 cm, Dosis 270 pro Minute. Die Bestrahlungsdosen wurden allmählich vergrössert und zwar in nachstehender Reihentolge: 125r, 250r, 500r, 1000r, 2000r, 4000r, 8000r, 12000r, 16000r, 24000r und 32000r. Die Aussaat geschah durch eine gleiche Anzahl von Samen für jede Dosis und in 6 Wiederholungsreihen einen Monat nach der Bestrahlung ²⁾ (16-IV-1934) in den Bedingungen eines warmen Treibebeckes ³⁾. Im Frühjahr 1935 wurden die erhaltenen Wurzelstöcke von den 1934 ausgesäten Samen in den Boden in Feldbedingungen verpflanzt und zwar ebenfalls in 6 Wiederholungsreihen. Bei der Umpflanzung wurde konstatiert, dass eine beträchtliche Menge des Auspflanzungsmaterials, d.h. der Wurzelstöcke Kontrolle und Varianten bei geringen Bestrahlungsdosen ergaben. In dem Masse wie die Dosen von 4000r ansteigen verringert

¹⁾ Der Botaniker F. N. RUSSANOW (6) hat den Kendyr des Amu-Darja Tals *Apocynum scabrum* RUS. genannt

²⁾ AFANASSJEW (2) weist auf Grund ihrer Versuche über die Einwirkung der X-Strahlen auf Weizensamen darauf hin, dass innerhalb eines Jahres der Zeitraum von der Bestrahlung bis zur Aussaat den Wirkungseffekt nicht herabsetzt.

³⁾ Der Versuch wurde im ersten und zweiten Jahr in Nordkirgisien, in der Tschui-Station (Zentralasien) des Instituts für Neuen Rohbast angestellt.

sich bei jeder nächstfolgenden Variante die Anzahl der Pflanzen und ihr Wurzelsystem wird schwächer. Wenn wir bei der Kontrolle die Zahl der Wurzelstöcke für 100% (149) annehmen, so ergibt die Dosis 4000r : 90,6% (135), 8000r : 75,8% (113), 16000r : 35,5% (53), 24000r : 13,4% (20). Geringe Dosen unter 4000 runterscheiden sich der Anzahl ihrer Wurzelstöcke nach wenig von der Kontrolle.

Aus den angeführten Angaben ist ersichtlich, dass grosse Röntgen-Dosen eine deprimierende Wirkung ausüben und die Anzahl der Pflanzen in den Reihen stark vermindern. Die Pflanzen gingen augenscheinlich unter der Einwirkung letaler Dosen noch im Stadium des Aufgehens zugrunde. Die der Keimung überlassenen bestrahlten Samen zeigten, dass sie bei verschiedenen Bestrahlungsdosen einen Keimungsprozentsatz aufwiesen, der sich von der Kontrolle fast nicht unterschied, so dass wir die geringe Anzahl von Pflanzen bei Varianten mit grossen Bestrahlungsdosen keineswegs dem schlechten Keimungsvermögen der Samen zuschreiben müssen. Ein Absterben der Pflanzen jedoch bereits im verhältnismässig erwachsenen Zustande haben wir nicht beobachtet mit Ausnahme einer geringen Anzahl (5-6) bei der maximalen Dosis 32000r.

BEOBSACHTUNGEN IM ERSTEN VEGETATIONSJAHR

Im Laufe des I. Vegetationsjahres wurden Beobachtungen über die Stärke des Vegetationswachstums und des Eintretens einzelner Entwicklungsphasen angestellt. Die Angaben phenologischer Beobachtungen über das Aufgehen im Treibhaus führen wir hier nicht an, da wir über keine genauen Angaben verfügen. Das kalte Frühjahr 1934 veranlasste uns das Treibebeet verdeckt zu halten, so dass einer regelrechten Kontrolle der Keimlinge nach den Wiederholungsreihen bei verschiedenen Varianten gewisse Schwierigkeiten entgegentraten.

Die meisten Pflanzen des Experiments gelangten im I. Vegetationsjahr nicht zur Blüte. Einzelne Blüten, die zu Ende der Vegetation bei einer geringen Anzahl von Pflanzen erschienen, gaben keine Früchte.

Dabei war bei allen Varianten des Experiments der Anzahl und dem Zeitpunkt des Auftretens der Knospen und Blüten nach kein Unterschied zu verzeichnen.

Ungefähr jede Dekade wurden Höhemessungen vorgenommen so-

wie die Anzahl der Blätter berechnet, um die Dynamik des Wachstums und der Anhäufung der Blätter festzustellen.

Aus der Analyse von Tabelle I ist ersichtlich, dass bereits von dem I. Messungsdatum an (11-VI) im Vergleich zur Kontrolle ein äusserst unbedeutendes Anwachsen der Höhe bei der Dosis 500r beobachtet wird und von dem II. Messungsdatum an (22-VI) bei der Dosis 2000r.

Den maximalen Unterschied erreicht die Dosis 500r jedoch beim letzten Messungsdatum — 18-VIII, und 2000r — am 6-VIII.

Wenn wir uns dem Wahrscheinlichkeitskoeffizienten des Unterschieds bei den zwei oben angeführten Dosen der drei Messungen (d. 13-VII, 25-VII und 6-VIII) zuwenden, so überzeugen wir uns von der 100⁰,-igen Wahrscheinlichkeit. Freilich, beim Vergleich der Angaben zweier Dosen kann man merken, dass bei 2000r der Wahrscheinlichkeitsprozentsatz höher ist (85–95⁰%) als bei 500r (60–65%). Zum letzten Termin der Wachstumsmessung jedoch (18-VIII) ändert sich das Bild: bei 500r erscheint der Unterschied vollkommen ausgeprägt

$$\frac{M_1 - M_2}{\sqrt{m_1 + m_2}} = 4,14$$
, bei 2000r wird sie bereits unzureichend (1,55).

Die Deprimierungserscheinungen beginnen mit 4000r und vergrössern sich in der Masse wie die Dosierung ansteigt, einer 100⁰,-igen Wahrscheinlichkeit des Unterschieds erreichen die Dosen von 12000r an (3,94).

Wenn wir uns nun dem Diagramm Nr. 1 zuwenden und die Kurven aller Messungsdaten einer Analyse unterziehen, kommen wir auch zu dem Schlusse, dass vom Beginn der Vegetation an bei geringen Dosen bis 2000r eine stimulierende Wirkung der X-Strahlen beobachtet wird. Natürlich können wir nicht behaupten, dass als Grenze der stimulierenden Wirkung die Dosis 2000r erscheint. Es lässt sich vermuten, dass die optimale Dosis der positiven Einwirkung der X-Strahlen irgendwo zwischen 1000 und 4000r liegt.

Von 4000r an lässt sich auf dem Diagramm mit Deutlichkeit eine gesetzmässige deprimierende Wirkung grosser Bestrahlungsdosen auf die Höhe verfolgen.

Die Angaben der Tabelle 2 zeigen, dass bei 500r fast vom Beginn der Vegetation eine Tendenz zum Ansatz von Blättern besteht und gegen Schluss der Vegetationsperiode erreicht diese Zunahme in

TABELLE 1. DURCHSCHNITTSHÖHE DER STENGEL IN CM ¹⁾

Dosierung	11/VI 1934	22/VI 1934	3/VII 1934	13/VII 1934	25/VII 1934	6/VIII 1934	18/VIII 1934
Kontrolle	4,36	6,98	12,31	16,85	21,16	25,26	27,12
125r	4,16	6,80	12,74	± 0,82 17,59	± 0,94 21,96	± 1,07 26,15	± 1,21 28,48
250r	4,10	7,08	12,26	17,48	21,75	± 1,04 25,96	29,59
500r	4,56	7,86	—	18,34	22,79	± 0,94 27,15	33,61
1000r	4,32	7,52	12,16	± 0,80 18,63	± 0,89 21,39	± 1,18 26,35	± 1,68 28,12
2000r	4,27	7,41	13,02	18,85	23,91	± 1,21 28,79	29,82
4000r	3,80	6,07	10,73	± 0,74 15,38	± 0,96 17,53	± 0,98 22,82	± 1,19 23,14
8000r	3,47	5,24	9,82	13,41	15,89	± 1,01 21,09	22,07
12000r	3,19	4,79	8,40	11,07	14,28	± 1,28 18,27	22,39
16000r	2,26	3,10	5,88	8,62	10,95	± 1,41 14,34	15,83
24000r	0,78	1,56	3,50	4,50	6,00	± 1,54 6,34	10,76

¹⁾ Zwecks Berechnung der Höhe und Anzahl der Blätterpaare nahmen wir im Durchschnitt von der Kontrolle an und bis 8000r incl. je 80 Stengel, bei den übrigen Dosen — alle vorliegenden Stengel. So bei 12000r — 52 St., bei 16000r — 42 Stengel, bei 24000 — 12 Stengel

Die Pflanzen der Dosis 32000r wurden im 1. Jahr nicht überprüft wegen zufälliger Beschädigungen, die sich bei einer geringen Anzahl von Exemplaren vorfanden.

Mathematisch bearbeitet wurden bloss die Angaben derjenigen Dosen, bei denen eine grosse stimulierende oder deprimierende Wirkung der X-Strahlen vermutet wurde.

Vergleich zur Kontrolle gegen 11%. Die Dosis 2000r unterscheidet sich jedoch nicht von der Kontrolle mit Ausnahme des Datums 6/VIII, wo sie eine Zunahme auf 15,7% erreicht. Die deprimierende Wirkung der X-Strahlen äussert sich auf den Blättern fast ebenso wie auf der Höhe der Pflanzen — mit der Vergrösserung der Bestrahlungsdosen, von 4000r an, fällt die Zahl der Blätter entsprechenderweise.

Unter der geringen Anzahl Pflanzen der letzten Bestrahlungsdosis

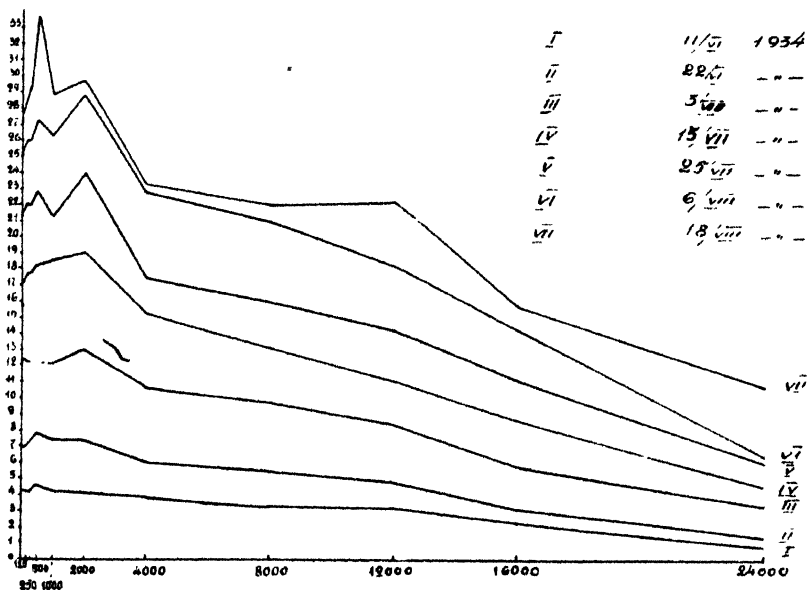


Fig. 1

stellten wir bei der letzten Bestrahlungsdosis 5-6 Pflanzen fest, die sich in morphologischer Hinsicht sehr bedeutend von den übrigen Exemplaren dieser Variante unterschieden. Wir geben hier ganz kurz die Beschreibung dieser Pflanzen. Sie zeichneten sich durch ihren Zwergwuchs aus, die Blätter hatten eine abgerundete Form, waren sehr klein, aber dafür sehr dick und dicht beflaumt. Ausserdem hatten die vorliegenden Pflanzen eine hellere Färbung der Blätter als die Kontroll Exemplare. Die Zwergexemplare gingen, nachdem sie das Stadium der 2-3 Paarblätter erreicht hatten, zugrunde.

TABELLE 2. DURCHSCHNITTSZAHL DER BLÄTTERPAARE AUF EINER PFLANZE (AUF DEM HAUPTSTENGEL)

Dosierung	11/VI	22/VI	3/VII	13/VII	6/VIII	18/VIII
Kontrolle	4,06	6,45	9,24	9,22	10,95	12,73
125r	4,35	6,99	9,29	10,62	10,70	12,16
250r	4,35	6,52	9,07	10,98	11,85	13,06
500r	4,70	7,13	8,85	10,15	12,25	14,13
1000r	4,28	5,93	9,20	11,57	10,49	12,47
2000r	4,03	6,19	9,21	9,52	12,99	12,89
4000r	3,81	5,44	8,21	9,16	10,05	12,42
8000r	3,71	5,50	8,05	9,15	9,42	10,40
12000r	3,46	5,04	6,59	7,10	8,65	10,06
16000r	2,89	3,54	5,44	6,51	6,54	7,87
24000r	0,77	2,25	3,22	4,62	4,44	6,44
32000r	—	—	—	—	—	—

BEOBACHTUNGEN IM II. VEGETATIONSJAHR

Im zweiten Vegetationsjahr wurden ebenfalls Berechnungen und Beobachtungen der Intensität des Wachstums (Höhe, Buschungsvermögen, Verzweigung) des Auftretens einzelner Entwicklungsstadien, der Beschädigung durch den *Septoria*-Pilz und der Hinfälligkeit der Stengel angestellt. Die phenologischen Beobachtungen ergaben, dass sich die Pflanzen beim Eintreten der entsprechenden Entwicklungsphasen ebenso verhielten wie im verflossenen Jahr. Die einzelnen Knospen und Blüten, die zum Schluss der Vegetation auftraten, ergaben keine Früchte, zwischen den einzelnen Varianten wurde kein Unterschied in bezug auf ihre Phenologie konstatiert.

Die Einwirkung der Bestrahlung im zweiten Vegetationsjahr äusserte sich in der deprimierenden Einwirkung der X-Strahlen auf die Höhe der Pflanzen. Von 2000r an ist die Durchschnittshöhe der Pflanzen im Vergleich zur Kontrolle herabgesetzt, von 16000r an erscheint die Differenz jedoch deutlich ausgesprochen und der Wahrscheinlichkeitskoeffizient vollkommen möglich. Eine stimulierende Wirkung geringer X-Dosen auf das Wachstum der Pflanzen ist jedoch nicht

erwiesen, mit Ausnahme der Dosis 250r, wo der Unterschied bestimmt unzureichend erscheint.

Aus den Angaben bezüglich der Verzweigungsfähigkeit ¹⁾ ist ersichtlich, dass die erwähnte Dosis sich von der Kontrolle der Anzahl der Zweige nach um 40% unterscheidet und von allen übrigen Dosen durch ihre Stärke. Die übrigen geringen Dosen zeichnen sich durch eine starke Verzweigung der Pflanzen aus. Grosse Dosen dagegen nähern sich der Kontrolle oder bleiben ein wenig hinter derselben zurück.

Die Wurzelstöcke des *Apocynum*, die aus dem Treibbeet aufs Feld verpflanzt wurden, haben gewöhnlich im ersten Lebensjahr einen grossen Prozentsatz von hinfälligen Stengeln. Wir konnten einen so bedeutenden Faktor nicht umgehen und deswegen haben wir dieses Merkmal extra nach den einzelnen Varianten verfolgt.

TABELLE 3. PRÜFUNG DER HÖHE, VERZWEIGUNG UND HINFÄLLIGKEIT DER STENGEL ²⁾

Dosierung	Durchschnittshöhe in cm	Durchschnittsverzweigung auf einer Pflanze	Prozentsatz widerstands- fähiger Stengel	Prozentsatz der halb- hinfälliger Stengel	Prozentsatz der hinfälliger Stengel
Kontrolle	48,26 ± 1,6	9,55 ± 0,5	38,51	9,53	51,56
125r	45,20	11,53	39,39	27,28	33,33
250r	51,80 ± 1,8	13,37 ± 0,6	45,00	30,83	24,17
500r	47,61	11,50	45,39	27,61	27,00
1000r	48,74	12,32	45,80	16,03	38,17
2000r	48,62	8,98	55,92	13,49	30,59
4000r	41,70	11,85	47,87	14,28	37,85
8000r	41,46	9,47	54,95	9,91	35,14
12000r	41,71	9,19	58,92	14,28	26,80
16000r	35,16 ± 2,5	8,67 ± 0,9	56,36	7,27	36,37
24000r	—	8,41	46,67	12,50	40,83
32000r	35,68	8,68	54,16	12,50	33,34

¹⁾ Der Faktor der Verzweigung hat eine grosse phenologische Bedeutung
Genetica XX

Der Buschigkeit nach ergeben die geringen Dosen positive Abweichungen, so unterscheidet sich die Dosis 125r merklich von der Kontrolle durch eine bedeutende Anzahl von Pflanzen mit 2 und 3 Stengeln und gegen 1% mit 6 Stengeln. Durch ihre Buschigkeit zeichnen sich ferner die Dosen 500r und 1000r aus, wobei die erste Dosis eine grosse Anzahl Pflanzen mit 2 und 3 Stengeln aufweist und die zweite Dosis — mit 2 und 3 Stengeln. Bei den übrigen lässt sich ein Zurückbleiben in bezug auf das Buschigkeitsvermögen beobachten mit Ausnahme der Dosis 24000r, wo 5% der Pflanzen mit 4 schwach entwickelten Stengeln festgestellt wurden.

TABELLE 4. BERECHNUNG DES BUSCHIGKEITSVERMÖGENS

Dosierung	Prozentsatz von Pflanzen mit einer Stengelanzahl von					
	1	2	3	4	5	6
Kontrolle	94,56	2,72	2,04	—	0,68	—
125r	86,37	6,36	5,45	—	0,91	0,91
250r	97,44	1,71	0,85	—	—	—
500r	92,22	3,54	2,83	—	1,41	—
1000r	91,98	1,78	4,46	—	1,78	—
2000r	97,25	1,37	0,69	0,69	—	—
4000r	97,78	1,48	—	0,74	—	—
8000r	100,00	—	—	—	—	—
12000r	96,24	1,88	1,88	—	—	—
16000r	98,13	1,87	—	—	—	—
24000r	95,00	—	—	5,00	—	—
32000r	100,00	—	—	—	—	—

Ehe wir uns der Darlegung der Ergebnisse der Ansteckung des *Apocynum*-Stengels mit dem *Septoria*-pilz, sei noch bemerkt, dass wir während der Vegetationsperiode eine zweimalige Bespritzung der

für die technologische Bewertung der Bastkulturen. Vom biologischen Gesichtspunkt jedoch erscheint die Verzweigung als positives Moment.

^{*)} Von der Kontrolle an und bis 4000r incl. nahmen wir für unsere Berechnung im Durchschnitt 140 Stengel für jede Variante, bei den übrigen Dosen je nach der Anzahl der vorliegenden Pflanzen.

So bei 8000r: 113 Stengel, 12000r: 56 St., 24000r und 32000r: 24 St.

Pflanzen mit der Bordeaux-Flüssigkeit vornahmen, die den Entwicklungsgrad der Pilze bedeutend herabsetzte. Das Ausmass der Ansteckung wurde nach dem 10 Befallssystem vorgenommen, und der Durchschnittsbefall für jede Variante festgestellt. In den Data der Tabelle 5 wird angefangen von der Kontrolle und bis 32000r fast kein Unterschied wahrgenommen. Die Reihen unterscheiden sich durch Zehntelbruchteile des Befalls, die beim Vergleich belanglos erscheinen.

TABELLE 5. ANSTECKUNGSGRAD DER APOCYNUM-STENGEL MIT DEM SEPTORIA-PILZ

Dosierung	Durchschnittsbefall der Ansteckung durch den <i>Septoria</i> -Pilz
Kontrolle	2,82
125r	2,85
250r	2,69
500r	2,90
1000r	2,21
2000r	2,72
4000r	2,65
8000r	2,85
12000r	2,67
16000r	2,39
24000r	2,60
32000r	2,16

Für die Kultur des *Apocynum venetum* L. ist es äusserst wichtig die Einwirkung der R-Strahlen auf die technologischen Merkmale festzustellen. Hierbei möchte ich erwähnen, dass es mir zuerst gelungen ist, unter der Einwirkung der X-Strahlen eine Veränderung der Länge und Feinheit der Elementarfaser zu erzielen.

Bestimmte Bestrahlungsdosen haben eine positive Einwirkung auf die Länge der Elementarfaser. So unterscheidet sich von 125r–500r und von 12000r–32000r die Länge der Elementarfaser im Vergleich zur Kontrolle durch 6,4–34,6%.

Die dazwischenliegenden Dosen von 1000r–8000r besitzen kürzere Fasern als die Kontrollexemplare. Der Feinheit der Faser nach unter-

scheiden sich alle bestrahlten Pflanzen von der Kontrolle durch eine grössere metrische Nummer, d.h. durch dünnere Fasern ¹⁾.

TABELLE 6. TECHNOLOGISCHE ANALYSE DES STENGELS ²⁾

Varianten	Durchschnitts- länge d. Elemen- tarfaser in mm.	länge der Elementarfaser in % der kontrolle	Metrische Nummer
Kontrolle	7,8	100	3641
125r	8,8	125,6	5660
250r	8,8	112,8	4674
500r	8,3	106,4	4780
1000r	6,9	88,4	4781
2000r	7,5	96,1	4312
4000r	6,4	82,0	5300
8000r	7,4	94,8	4389
12000r	8,9	114,1	5419
16000r	8,4	107,7	6132
24000r	8,3	106,4	5872
32000r	10,5	134,6	5596

ZUSAMMENFASSUNG

1) Die Beobachtungen betreffs des Eintretens einzelner Entwicklungsphasen bei *Apocynum venetum* L. haben erwiesen, dass bei den angewandten Varianten der X-Strahlen es uns nicht gelungen ist, die Zeit des ganzen Entwicklungszyklus abzukürzen.

2) Im ersten Vegetationsjahr haben geringe Dosen der X-Bestrahlung eine unbedeutende positive Wirkung auf die Stärke des vegetativen Wachstums des *Apocynum*. Beträchtlichere Dosen, von 4000r an, haben eine deprimierende Wirkung und bei 32000r erscheinen verunstaltete und Zwergexemplare (vielleicht Mutanten).

3) Im zweiten Vegetationsjahr äussert sich die Wirkung der Röntgenstrahlen in der deprimierenden Wirkung grosser Dosen auf das

¹⁾ Die metrische Nummer bedeutet das Verhältnis der Länge der Faser zu ihrem Gewicht.

²⁾ Der Ernte des Herbstes 1935

Wachstum der Pflanzen. Pflanzen, die mit geringen Dosen bestrahlt wurden, wiesen im Vergleich zu den grossen Dosen und zur Kontrolle ein grösseres Verzweigungsvermögen auf. Äusserst klar tritt die positive Einwirkung geringer Dosen auf die Buschigkeit der Pflanzen zutage, sowie die Wirkung aller Dosen auf die Senkung des Prozentsatzes hinfälliger Stengel. Die stimulierende Wirkung der Dosen 500r und 2000r, die im ersten Jahr konstatiert wurde, konnte im zweiten Jahr nicht festgestellt werden.

4) Bestimmte Bestrahlungsdosen üben eine positive Wirkung auf die Länge der Elementarfaser aus, desgleichen wirken alle Bestrahlungsdosen auf die Feinheit der Faser ein.

Zum Schluss möchte ich noch den Mitarbeitern unseres Instituts, L. P. BRESLAWETZ und G. B. MEDWEDEWA, die mir bei meiner Arbeit mit ihrem Rat beigestanden haben, meinen innigsten Dank aussprechen.

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THE LOCATION OF THE GENE FOR HAEMOPHILIA

by

J. B. S. HALDANE

Department of Biometry, University College, London

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Ever since MORGAN (1913) explained human sex-linkage, it has been generally believed that haemophilia was caused by a recessive gene in the X chromosome. On this hypothesis HALDANE (1935) concluded that the gene arises in man with a frequency of about once in 50,000 generations by a process of mutation. If new genes for haemophilia can only appear in the X chromosome by mutation, this estimate of the frequency follows from the fact that haemophilic males rarely live long enough to breed. And as about one third of the genes for haemophilia in the X chromosome are carried by males, the frequency of the gene in the X chromosome must be reduced by about one third in each generation, if it is not kept constant by new genes arising by mutation or some other process. This mutation (as HALDANE interpreted it) is not uncommon. In a recent study by BELL and HALDANE (1937) in which the linkage between the genes for colour-blindness and haemophilia was demonstrated, the gene for haemophilia had occurred anew in three out of the six families studied. The rarity of such cases in the literature was explained by the fact that interest in haemophilia had been largely confined to cases where a number of members of a family were affected.

In a most interesting paper SIRKS (1937) has suggested that the gene for haemophilia (h) and its normal allelomorph (H) may occur both in the X and the Y chromosome, and that the new appearance of h in X chromosomes is due not to mutation but to crossing over. It is the object of this paper to show that the frequency of crossing

over can be roughly estimated, and to examine other consequences of SIRKS' hypothesis.

On this view haemophilia would be analogous to *bobbed* in *Drosophila melanogaster*, and to a group of genes in Cyprinodont fishes studied by AIDA (1921), WINGE (1934), and others. HALDANE (1936) has brought forward evidence that six human genes can occur in both the X and the Y chromosomes, namely the genes for dominant and recessive forms of retinitis pigmentosa (in certain pedigrees only) for achromatopsia, xeroderma pigmentosum, Oguchi's disease, and epidermolysis bullosa dystrophica. In these cases, however, crossing over is common, and the frequency of the genes in the X and Y chromosomes must be about equal. On SIRKS' theory the gene for haemophilia must be very common in the human Y chromosome, but very rare in the X. This condition was found by de ZULUETA (1925) in the beetle *Phytodecta variabilis* where crossing over between the X and Y chromosome is very rare or absent. The origin of h in the X chromosome, instead of being due to gene mutation, as HALDANE assumed, may, on SIRKS' theory, be compared with certain of the mutations of *Oenothera*, which are due to an unusual type of crossing over between chromosomes which carry different genes.

On SIRKS' theory there are three female genotypes, namely

$\frac{XH}{XH}$, $\frac{XH}{Xh}$, and $\frac{Xh}{Xh}$, and four male genotypes, namely $\frac{XH}{YH}$, $\frac{XH}{Yh}$, $\frac{Xh}{YH}$, and $\frac{Xh}{Yh}$. $\frac{Xh}{Yh}$ males are haemophilic. $\frac{Xh}{Xh}$ females are so rare

that we do not know their phenotype. SIRKS further assumes that in $\frac{XH}{Yh}$ (and presumably therefore also in $\frac{Xh}{YH}$) males crossing over occurs with a small frequency, which he thinks might be as high as

$1/2\%$. Thus $\frac{XH}{Yh}$ males yield a large majority of XH and Yh gametes,

but also a few Xh and YH.

One difficulty of SIRKS' theory is as follows. As HALDANE (1935) pointed out, and as also appears from the studies of BIRCH (1937) there are several allelomorphs of h. One of them, which we may call h', determines a type of haemophilia which though troublesome is rarely

fatal. We must thus assume that the human Y chromosome, though normally carrying h, sometimes carries h'.

The main objection, which, as we shall see, is not insuperable, is the following. If crossing over had anything like the frequency which SIRKS assumes ($1/2\%$ or over), H genes would continually cross over into the Y chromosomes from the X. Now it is known that about half the sons of the daughters of haemophiles are haemophilic. If H genes were common in the human Y chromosome this would not be so. We shall see that this objection can be met if the frequency of crossing over is sufficiently small, and certain other assumptions are made.

For this purpose a simple mathematical analysis is necessary. We shall consider a large population in which there is no inbreeding, and generations are kept separate. An allowance for overlapping of generations, and for occasional cousin marriage greatly complicates the analysis, but does not appreciably affect its result. We shall further

make (with SIRKS) the simplifying assumption that no $\frac{Xh}{Yh}$ males

survive to breed. We shall assume the same for $\frac{Xh}{Xh}$ females, though

this restriction will later be removed.

Suppose that the nth generation is formed from gametes in the proportions:

Eggs	u_n	XH : 1 Xh
X-spermatozoa	v_n	XH : 1 Xh
Y-spermatozoa	w_n	YH : 1 Yh

Let p be the small frequency of crossing over, and $p + q = 1$, so

that $\frac{XH}{Yh}$ males form gametes in the proportions:

$$q \text{ XH} : p \text{ Xh} : p \text{ YH} : q \text{ Yh},$$

and $\frac{Xh}{YH}$ males in the proportions:

$$p \text{ XH} : q \text{ Xh} : q \text{ YH} : p \text{ Yh}.$$

The nth generation consists of:

$$u_n v_n \frac{XH}{XH} : (u_n + v_n) \frac{XH}{Xh} : 1 \frac{Xh}{Xh} \quad \&$$

$$u_n w_n \frac{XH}{YH} : u_n \frac{XH}{Yh} : w_n \frac{Xh}{YH} : 1 \frac{Xh}{Yh} \quad \delta$$

These zygotes form gametes in the proportions:

$$\begin{aligned} \text{Eggs} & \dots (2u_n v_n + u_n + v_n) XH : (u_n + v_n) Xh \\ \text{X-spermatozoa} & \dots (u_n w_n + qu_n + pw_n) XH : (pu_n + qw_n) Xh \\ \text{Y-spermatozoa} & \dots (u_n w_n + pu_n + qw_n) YH : (qu_n + pw_n) Yh \end{aligned}$$

Hence we have the equations:

$$\begin{aligned} u_{n+1} &= \frac{2u_n v_n + u_n + v_n}{u_n + v_n} \\ v_{n+1} &= \frac{u_n w_n + qu_n + pw_n}{pu_n + qw_n} \\ w_{n+1} &= \frac{u_n w_n + pu_n + qw_n}{qu_n + pw_n} \end{aligned}$$

If $\frac{Xh}{Xh}$ females are viable the first of these becomes

$$u_{n+1} = \frac{2u_n v_n + u_n + v_n}{u_n + v_n + 2}.$$

As however u_n and v_n are large this correction is unimportant.

The frequency of haemophilia among male births is $\frac{1}{(1+u_n)(1+w_n)}$.

But in London this lies between 4×10^{-5} and 2×10^{-4} (HALDANE 1935), and may be taken as roughly 10^{-4} . Now w_n is a small fraction, for most human Y chromosomes carry h. Hence $u_n \approx 10,000$ approximately. We shall use this number, remembering that the true value is probably a little higher, but may be anywhere between 25,000 and 5,000.

Now the population is roughly in equilibrium. The frequency of haemophilia is not rising or falling very rapidly. It follows that u_n and v_n are approximately equal and constant. Hence

$$u_n = v_{n+1} = \frac{u_n w_n + qu_n + pw_n}{pu_n + qw_n} \text{ approximately.}$$

But p is small. Hence $u_n = \frac{u_n w_n + u_n}{pu_n + w_n}$ approximately.

Hence $w_{n+1} = w_n + pu_n$, or $p = \frac{1}{u_n} = 10^{-4}$ approximately. If

we allow for the fact that not all haemophilics die, but their fertility is about $1/4$ of the normal, the value of p will be reduced by about $1/4$. In fact the cross-over value on SIRKS' theory is three times the mutation rate on HALDANE's theory, since according to SIRKS new h genes can only appear in the X chromosomes of males, whilst according to HALDANE they can appear in the X chromosomes of females also.

On SIRKS' theory the population cannot be in complete equilibrium. For it follows from equations (1) that

$$\begin{aligned} w_{n+1} - w_n &= \frac{pw_n(u_n - w_n) + pu_n + qw_n}{qu_n + pw_n} \\ &= p(1 + 2w_n) \text{ approximately.} \end{aligned}$$

$$\text{Hence } 2w_{n+1} + 1 = (1 + 2p)(2w_n + 1)$$

$$\text{and if } w_0 \text{ is zero, } w_n = 1/2 [(1 + 2p)^n - 1]$$

$$= 1/2 (e^{2np} - 1) \text{ approximately.}$$

Thus after 1,000 generations (say 25,000 years) w_n would reach the value 0.11, and after 10,000 generations (say 250,000 years) w_n would rise to 3.2. Clearly, then, the crossing over of H genes into the Y chromosome as demanded by SIRKS' theory cannot have been going on for as long as 10,000 generations. In its original form this theory implies two assumptions.

1) By some unknown process the Y chromosomes of primitive men or their animal ancestors were completely or almost completely furnished with h genes (and a few h' genes) while very few of their X chromosomes were so provided.

2) The frequency of crossing over is now about 10^{-4} , but was very much less until somewhere in palaeolithic times. If the rate were not now so large, haemophilia would be rarer than it is. If it had been so large for 10,000 generations, heterozygous women would rarely bear haemophilic sons.

I submit that these hypotheses are less plausible than HALDANE's hypothesis that a certain normal gene in man mutates at a rate slightly greater than certain normal genes in *Drosophila*. Nevertheless in fairness to SIRKS it must be pointed out that a small addition to his

theory will save it. Suppose that $\frac{XH}{Yh}$ (and possibly $\frac{Xh}{YH}$) men are very

slightly fitter, say $(1 + k)$ times fitter, than $\frac{XH}{YH}$ men. The extra

fitness might be due to greater vitality of greater fertility resulting in their begetting, on the average, $(1 + k)$ times as many children.

Then

$$w_{n+1} - w_n = w_n (2p - k) + p,$$

and w_n tends to a constant value $\frac{p}{k - 2p}$, provided $k > 2p$. Thus if

$k = 10p = 10^{-3}$, w_n would be stabilized at $1/8$, which is quite consistent with existing knowledge. This subsidiary hypothesis explains, first, how by selection, even if mutation were very rare, the majority of Y chromosomes came to carry h, and secondly, why this condition is permanent in spite of crossing over.

The exceedingly low value of p is however a difficult feature in SIRKS' theory, especially if HALDANE's evidence for the existence of 6 other incompletely sex-linked genes with much higher cross-over values be accepted.

Other more serious difficulties are as follows. There must by some YH chromosomes, even if they are rare compared with Yh. Occasionally then a $\frac{XH}{Xh}$ woman must have sons by a $\frac{XH}{YH}$ man. These sons are

$\frac{Xh}{YH}$, and must transmit haemophilia to their daughters' sons. Thus

we should expect occasional transmission through unaffected males. This has not yet been observed. Again the gene h' must not be very

uncommon in the Y chromosome. $\frac{Xh}{Yh'}$ males presumably show hae-

mophilia to a slight extent, like $\frac{Xh'}{Yh}$ males. We should therefore

expect an occasional fraternity of mild haemophilics in a family most of whose members have severe haemophilia. Until SIRKS can produce examples of these phenomena his theory will remain open to severe criticism.

Two types of evidence should enable geneticists to decide between the two rival theories. In the first place if the origin of haemophilic genes in the X chromosome is studied, it may be possible to determine whether they appear only in the spermatogenesis (SIRKS) or in oögenesis also (HALDANE). Thus if a woman bore 4 colour-blind non-haemophilic sons, 4 normal sons, and one haemophilic, this would involve, on SIRKS' theory, not only a very abnormal segregation in a heterozygote but also four or five cross-overs between the loci of colour-blindness and haemophilia, if she was heterozygous for both. If x is the frequency of crossing over between the loci of colour-blindness and haemophilia this has a probability slightly exceeding

$$\frac{315x^4(1-x)^5}{256} \text{ or if } x = \text{about } 0.05 \text{ as BELL and HALDANE conclude, a}$$

probability of about $6 \cdot 10^{-6}$, whereas such a case could readily be explained by mutation. So far no cases have been described inconsistent with SIRKS' theory.

Secondly it may be hoped that BELL and HALDANE's work will be followed up, and the human X chromosome mapped. If the gene for haemophilia can be shown to lie between two ordinary sex-linked genes, say those for colour-blindness and for major ectodermal defect (anidrosis) SIRKS' hypothesis will become untenable. If on the other hand the locus of haemophilia, like that of *bobbed*, lies at one end of the chromosome map, SIRKS' hypothesis will receive powerful support.

At present there is no cogent evidence for accepting one theory rather than the other, although, to the writer at least, the mutation theory seems to have the merits of greater simplicity and a closer accord with the known facts.

SUMMARY

SIRKS' theory that the gene for haemophilia can cross over between the X and Y chromosomes is critically examined. The cross-over frequency must be of the order of one in ten thousand. Some difficulties in the theory are pointed out, and methods by which it may be tested are discussed.

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A CONTRIBUTION TO A CYTOGENETICAL SURVEY OF THE MALVACEAE

by

C. E. FORD

Botany Department, University of London, King's College

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INTRODUCTION

The present work is a continuation of the cytological study of the Malvaceae begun at King's College by Dr. J. H. DAVIE under the direction of Professor R. R. GATES. Chromosome counts in 32 species of the family are reported and are summarised in Table I. The general observations are described under the relevant genera, which are arranged in the order adopted in the „Pflanzenfamilien“ of ENGLER and PRANTL. A short discussion of the distribution of chromosome numbers and its significance in each tribe follows the account of observations made on species of that tribe. Although no representatives of the Malopeae have been examined, a similar discussion of the known chromosome numbers in this tribe is added for the sake of completeness.

DAVIE (1933, 1935), and SKOVSTED (1935c) are the principal contributors to the knowledge of chromosome numbers in the Malvaceae. Their lists have been used freely in compiling tables 2, 3 and 4. References to all publications containing the original reports of chromosome numbers of species in the family are included in the bibliography and are marked with an asterisk.

Apart from the distribution of chromosome numbers, the principal observations of general interest concern intraspecific variation in chromosome number. A full discussion of this subject is appended.



ORIGIN OF MATERIAL AND TECHNICAL METHODS EMPLOYED

Seeds were received from Botanic Gardens both at home and abroad and plants were grown at the Courtauld Laboratory, Regent's Park, in the summers of 1934, 1935 and 1936.

Wherever possible, herbarium specimens were prepared and taken to Kew for identification. In some cases the herbarium sheets were replaced or supplemented by fresh material. Root tips were fixed in Navashin, Benda or La Cour 2 BD, while for meiotic stages the staminal columns were dissected out from young buds, dipped in Carnoy and fixed in La Cour 2 BE, Medium Flemming or a modified Medium Flemming containing uranium trioxide in place of osmic acid.

Material was washed, dehydrated, and embedded according to La Cour's schedule (LA COUR 1931). Sections were cut at 10-20 μ , bleached, where necessary, on a hot plate for about 12 hours in a mixture of 40 cc. 95% alcohol and 20 cc. 20 volume hydrogen peroxide, and stained by Newton's Gentian Violet Iodine method.

Fixation of anthers in 3 : 1 acetic alcohol followed by crushing in aceto carmine gave satisfactory results in some cases.

OBSERVATIONS

a) Tribe *Malopeae*

Seven determinations (DAVIE 1933, SKOVSTED 1935) in 6 species show that the diploid numbers 44, 50, and possibly 42, occur in this tribe. These numbers are indicative of secondarily balanced polyploidy probably derived from the hexaploid 42 of the -7series which, as will be shown below, is dominant in the tribes Malveae and Ureneae.

It is of interest to note that according to SKOVSTED (1935) strains of *Malope trifida* exist with $2n = 44$, and $2n = 50$ respectively.

A similar variation occurs in *Anoda cristata* (see below). The phenomenon is of considerable interest and will be referred to in the discussion.

b) Tribe *Malveae*

A b u t i l o n

Counts were obtained from seven species all previously unexamined. Five of these are diploids with $2n = 14$, (figs. 1-6), while of the other two, one is definitely hexaploid with $n = 21$ and the other

TABLE 1. — CHROMOSOME NUMBERS IN THE SPECIES EXAMINED

Fig	Name given by Kew	Received as	Source of material	Ch. number	
				2n	n
1	Not identified	<i>Abutilon asiaticum</i>	Peradeniya, Ceylon	14	—
2, 3	<i>Abutilon auritum</i> SWEET	<i>A. auritum</i>	" "	14	—
4, 7	Not identified	<i>A. graveolens</i>	" "	14	7
5	" "	<i>A. hirtum</i>	Cienfuegos, Cuba	14	—
6	" "	<i>A. molissimum</i>	Peradeniya, Ceylon	14	—
	" "	<i>A. pauciflorum</i>	Cienfuegos, Cuba	ca 42	—
	" "	<i>A. Theophrasti</i>	Berlin	—	21
8	<i>Lavatera cachemiriana</i>	<i>Lavatera cachemiriana</i>	Dr J H DAVIE	—	22
	<i>L. cretica</i>	<i>L. cretica</i>	Ed Gdns, Swansea	—	c. 56
9	<i>Althaea officinalis</i> var ?	<i>Malva hirsuta</i>	" " "	—	21
	<i>Malva crispa</i> L.	<i>Malva crispa</i>	Roy Bot. Gdns, Kew	—	c. 56
10	<i>M. Alcea</i> L.	<i>M. Alcea</i>	" " " "	—	42
15	<i>Sidalcea candida</i> A GRAY	<i>Sidalcea candida</i>	" " " "	—	10
	<i>S. campestris</i> GREENE	<i>S. malvaeflora</i>	Ed Gdns, Swansea	—	10
11-14	<i>S. parviflora</i> GREENE	<i>S. orangea</i>	Roy Bot. Gdns, Kew	—	10
	<i>Napaea dioica</i> L.	<i>Sida napaea</i>	" " " "	—	14
	Not identified	<i>Sida carpinifolia</i>	Peradeniya, Ceylon	—	14
16-18	<i>Anoda cristata</i> L.	<i>A. cristata</i>	Berlin	30	15
19, 20	<i>A. cristata</i> L.	<i>A. hastata</i>	Roy Bot. Gdns, Kew	30	15
	<i>A. cristata</i> L.	<i>A. Wrightii</i>	Chelsea Physic Gdn	—	18
21	<i>Urena lobata</i> L.	<i>Urena lobata</i>	Dr J H DAVIE	28	—
	Not identified	<i>Pavonia fruticosa</i>	" "	28	—
22	" "	<i>Hibiscus angulosus</i>	Peradeniya, Ceylon	56	—
	" "	<i>H. cannabinus</i>	" "	ca 72	—
23	" "	<i>H. collinus</i>	" "	42	—
	<i>H. costatus</i>	<i>H. costatus</i>	Cienfuegos, Cuba	36	—
	Not identified	<i>H. diversifolius</i>	Kirstenbosch, S Africa	—	c. 72
	" "	<i>H. esculentus</i>	Cienfuegos, Cuba	—	c. 66
	" "	<i>H. esculentus</i>	Peradeniya, Ceylon	ca 66	—
	" "	<i>H. peduncularis</i>	Kirstenbosch, S Africa	ca 72	—
25	" "	<i>H. vitifolius</i>	" "	—	33
24	" "	<i>H. Trionum</i>	" "	28	—
26	" "	<i>Thespesia lampas</i>	Cienfuegos, Cuba	26	—
	" "	<i>T. populnea</i>	" "	26	—
	" "	<i>Gossypium Aimouria-num</i>	Dr. J M. WEBBER	26	—
	" "	<i>G. Harknessii</i>	" "	26	—

probably hexaploid with $2n = c. 42$. At mitotic metaphase in the diploid species it is possible to identify certain of the pairs of homologous chromosomes by characteristic features of their morphology. For instance, in *A. auritum* (figs. 2 and 3) the chromosomes of the complement may be distinguished as follows:

Pair A. Long, median centromere.

„ B. Long, sub-median centromere, sub-terminal secondary constriction.

„ C. Medium length, median centromere, satellite.

„ D. Short, sub-median centromere.

„ E. |

„ F. | Short, median centromere.

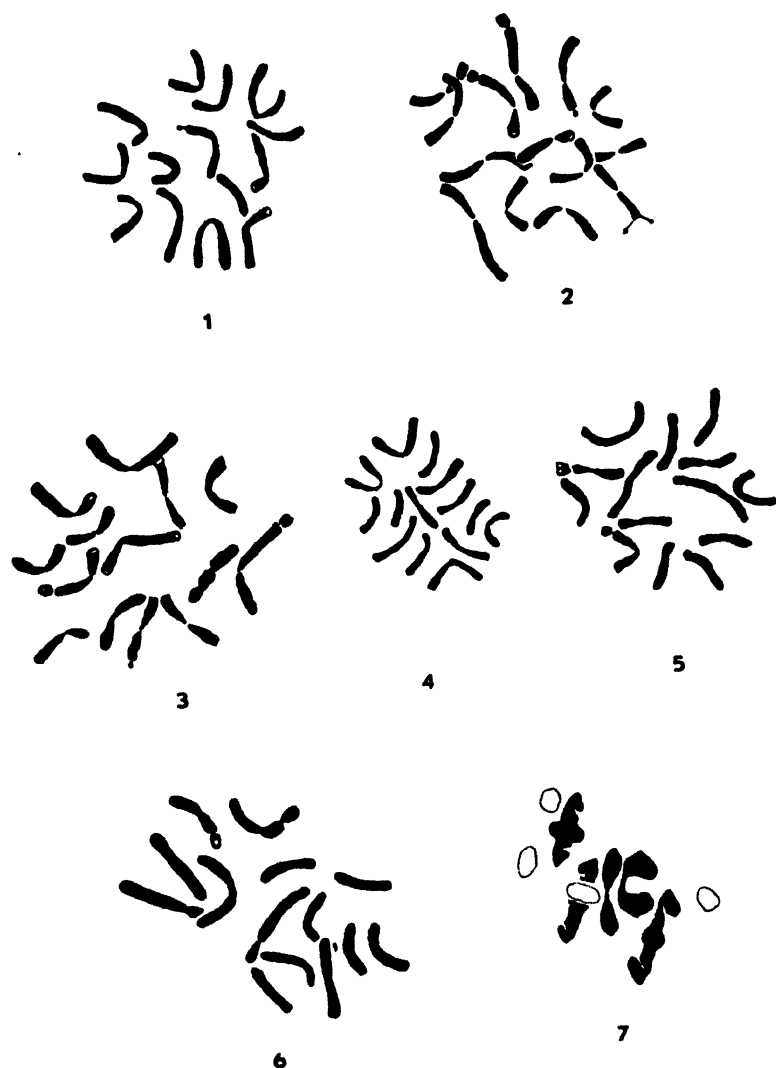
„ G. |

The long pairs A and B are about 3.3μ long while the shortest pair measure about 1.9μ . The chromosomes of the other diploid species examined, with the exception of *A. hirtum*, show approximately the same range of length. All were fixed in 2 BD. The counts in *A. hirtum* were made in a young petal initial fixed in uranic medium Flemming and here the longest and shortest chromosomes measured approximately 2.0μ and 1.3μ respectively.

Attachment of chromosomes to the nucleolus during mitotic prophase was observed in three of the diploid species, namely *A. asiaticum*, *A. auritum* and *A. mollissimum*. Two chromosomes (one pair) were probably involved in each case. The remaining species were not examined for nucleolar chromosomes.

In a preparation of pollen mother cell meiosis in *A. graveolens* there was a single loculus at metaphase I, the remainder having all reached the tetrad stage. Seven bivalents were most frequently observed, but in a total of 30 pollen mother cells, examined there were 22 univalents. This is, of course, an extremely high frequency of univalents for a simple diploid, and is indicative of some abnormality, structural or physiological, affecting chromosome pairing. A side view of metaphase I showing five bivalents and four univalents is figured (fig. 7). Three lines of evidence suggest that a physiological upset affecting this loculus alone was responsible for the high frequency of univalents observed.

- 1) Material was collected at the end of the flowering period (late October).



FIGS. 1-7. *Abutilon* spp. FIG. 1. *A. asiaticum*, metaphase, root tip mitosis. FIGS. 2 and 3. *A. auritum*, metaphase, root tip mitosis. FIG. 4. *A. graveolens*, metaphase, root tip mitosis. FIG. 5. *A. hirtum*, metaphase, mitosis in petal initial. FIG. 6. *A. mollissimum*, metaphase, root tip mitosis. FIG. 7. *A. graveolens*, metaphase I showing 5 bivalents and 4 univalents. All $\times 4700$.

- 2) Meiosis in this one locus evidently had either proceeded more slowly or had commenced later than in the other loculi.
- 3) The tetrads in the other loculi were normal. They did not contain micronuclei and microcytes were not observed. This suggests that meiosis in these loculi had been normal.

The metaphase chiasma frequency determined from 14 cells in side view was 1.36 per bivalent, while the terminalisation coefficient was 0.62. These values are lower than those determined in other species of the tribe (cf. *Anoda* and *Sidalcea* below) and, in view of the high frequency of univalents, are probably lower than the normal for this species.

Lavatera

Twenty-two bivalents were frequently observed at metaphase I in pollen mother cells of *L. cachemiriana*. This agrees with DAVIE's (1933, 1935) counts ($n = 22$) on material from the same source, but not with SKOVSTED's (1935) count of $2n = 42$. There is not the slightest doubt that my count of $n = 22$ is correct. Hence strains with different haploid numbers may occur in this species, though it is possible that SKOVSTED may have overlooked two chromosomes in his material, especially as the mitotic chromosomes are small and their number high. A certain amount of secondary association probably occurs at metaphase, but is not very marked (fig. 8). DAVIE (1935) reports loose secondary association in this species.

A few counts only at metaphase I in *L. cretica* were possible. These indicated that the haploid number was approximately 56, which agrees with SKOVSTED's (1935) count of $2n = c. 112$ in root tip mitosis.

Althaea

A single species of *Althaea*, identified as *A. officinalis* L. var? by Kew, was examined. Several counts at metaphase I showed that $n = 21$. Secondary association was observable but was not very marked (fig. 9).

Malva

Preparations of pollen mother cell meiosis showed that the haploid

numbers in *M. crispa* and *M. Alcea* were c. 56 and 42 respectively. A polar view of metaphase II in *M. Alcea* is illustrated (fig. 10). These counts confirm those made by SKOVSTED on these two species.

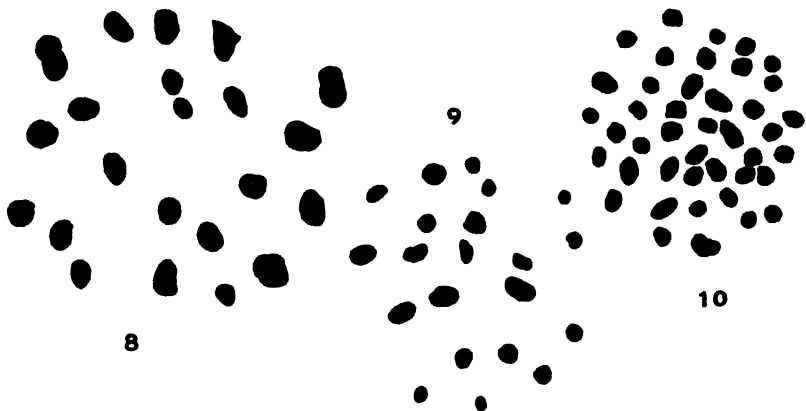


FIG. 8 *Lavatera cachemiriana*, metaphase I, polar view FIG. 9 *Althaea officinalis* var., metaphase I, polar view FIG. 10 *Malva Alcea*, metaphase II

Sidalcea

The haploid number 10 was observed in the three species examined *S. candida* A. GRAY, *S. campestris* GREENE, and *S. parviflora* GREENE.

S. parviflora was most thoroughly examined. At late diplotene the bivalents show differential contraction, not only between different bivalents, but also between different parts of a single bivalent (fig. 11). In the latter cases the relatively uncontracted part of the bivalent is generally distal to a chiasma. The two chromosomes of such bivalents are symmetrical as regards degree of contraction.

The late diplotene chiasma frequency and terminalisation coefficient were determined as 1.72 and 0.79 respectively from observations of 20 nuclei. Several bivalents with three chiasmata were seen at this stage. The exchange of partners between chromatids was clearly visible at some chiasmata. Finally, relational coiling between the chromosomes of a bivalent was observed occasionally. These coils can always be distinguished from the chiasmata by careful focussing.

A nucleolar bivalent with two satellites attached to the nucleolus is clearly visible at diplotene in the great majority of nuclei (figs. 12 and 13). Such bivalents only rarely have chiasmata in the proximal arm.

Both true and false interlocking occur frequently in the diplotene nuclei. A complex case is illustrated in Fig. 11. Here two ring bivalents are independently interlocked with the nucleolar bivalent, which as usual, has a single chiasma in the distal arm (obscured in the figure). When the nucleolus disappears during diakinesis such interlocks may be resolved and so they must be regarded as false interlocks. A false interlock between a rod bivalent and a ring bivalent is shown in the same figure.

Analysis of side views of metaphase I in 17 pollen mother cells gave an average chiasma frequency per bivalent of 1.63 and a terminalisation coefficient of 0.83. One of these nuclei is shown in Fig. 14. Comparison with the corresponding values for late diplotene indicates that a certain amount of terminalisation of chiasmata occurs between these two stages. This view is supported by the fact that no bivalents with three chiasmata were observed at metaphase, though, as stated above, such bivalents were not uncommon at diplotene.

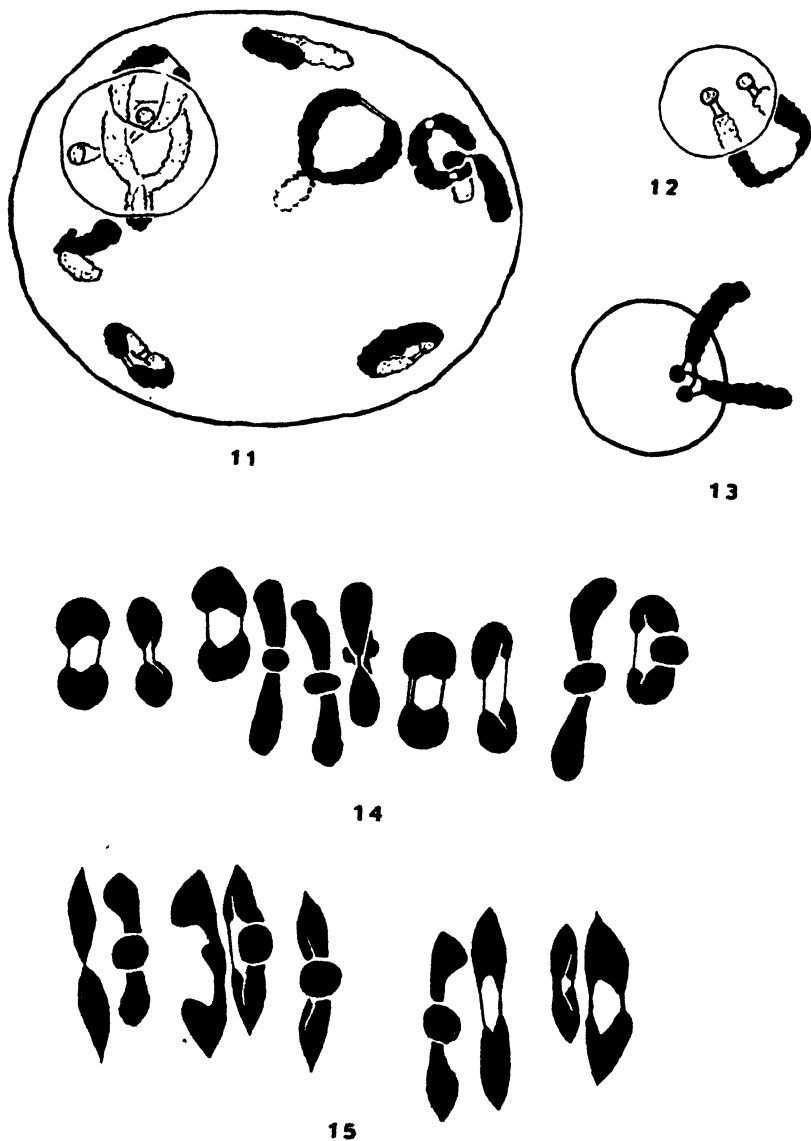
Secondary association was not observed in polar views of metaphase I.

In *S. campestris* the bivalents at metaphase I were very similar as regards size and form to those of *S. parviflora*, both in side and in polar view. There was no evidence of secondary association either at metaphase I or at metaphase II. Anaphase II chromosomes with median non-staining regions exactly like those described in *Anoda cristata* (below) were observed. Stages earlier than metaphase I were not available.

The bivalents at metaphase I in *S. candida* also resembled those of *S. campestris* and *S. parviflora* very closely. Nine metaphase I bivalents from a cut cell are figured (fig. 15). The metaphase chiasma frequency was 1.56 per bivalent and the terminalization coefficient 0.67. These figures were determined from 10 nuclei in side view and are rather lower than those determined for *S. parviflora*. A nucleolar bivalent was present in the only diplotene nucleus observed. Again, secondary association was not seen.

N a p a e a

Counts at metaphase I showed that 14 is the haploid number in *N. dioica*. This confirms the mitotic counts of $2n = 28$ made by SKOVSTED and by DAVIE on *Sida napaea* (= *N. dioica*).



FIGS. 11-15. *Sidalcea* spp. FIG. 11. *S. parviflora*, nucleus at diplotene showing differential contraction between parts of a bivalent and interlocking. FIGS. 12 and 13. *S. parviflora*, nucleolar bivalent at late diplotene. FIG. 14. *S. parviflora*, metaphase I, side view. FIG. 15. *S. candida*, metaphase I, side view from a cut cell. (9 bivalents only shown). FIGS. 9 and 10 $\times 3100$. Remainder $\times 4700$.

S i d a

One species alone, *S. carpinifolia*, was examined. Fourteen bivalents were counted at metaphase I.

A n o d a.

Seeds under the names *Anoda cristata*, *A. hastata*, and *A. Wrightii* were received from three different Botanical Gardens, but plants raised from them were all identified as *Anoda cristata* L. by Kew. They will be referred to as strains 1, 2 and 3 respectively.

Strain 1. Several counts at metaphase of root tip mitosis showed $2n = 30$. All the chromosomes had median or sub-median centromeres. No satellited chromosomes were observed.

At metaphase I of meiosis 15 bivalents were counted. The average chiasma frequency determined from 7 nuclei at this stage was 1.4 per bivalent while the terminalization coefficient was 0.85. A side view of metaphase is illustrated in Fig. 16. In polar view of metaphase I there is some indication of secondary association. The plate illustrated (fig. 17) could be interpreted as 2 associations of 2, 1 association of 3, and 8 free bivalents.

At the beginning of anaphase II, the daughter chromosomes have very prominent median non-staining regions (fig. 18). These gaps give a very characteristic appearance to the complement, both in polar and in side view.

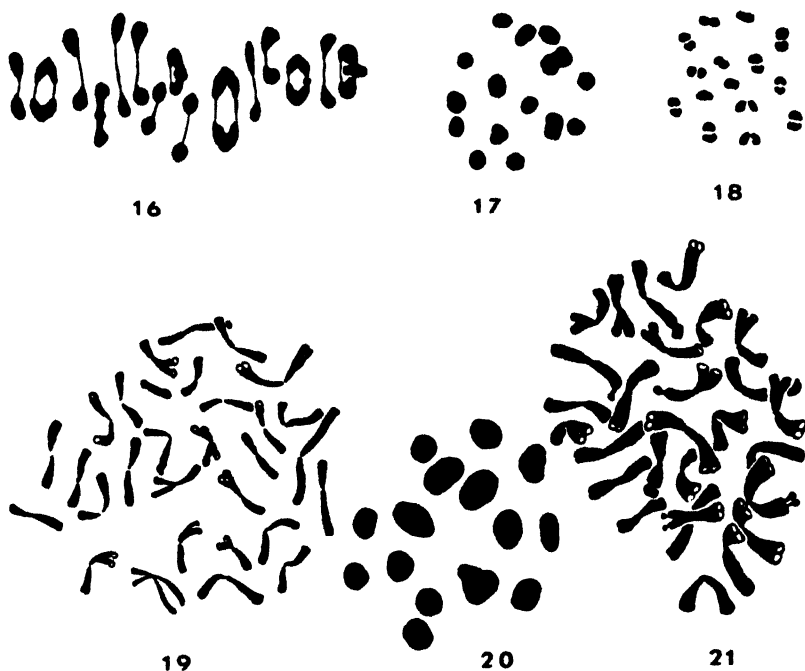
Strain 2. A somatic plate from a root tip fixed in Benda is figured (fig. 19). The chromosomes number 30, have median or sub-median centromeres, and range from $3.0\ \mu$ to $1.5\ \mu$ in length. No satellites were seen.

Counts of 15 bivalents were made at diakinesis and metaphase I of pollen meiosis. Secondary association occurs here also, though it is not very marked. A polar view of metaphase I in which there is probably one association of 3, and one association of 2 bivalents is illustrated (fig. 20). This nucleus might also be interpreted as showing one association of 3, five associations of 2, and 2 free bivalents.

Strain 3. This strain was received as *A. Wrightii* and was the material used by DAVIE (1935), who determined that the diploid number was 36. Several counts of 18 bivalents were made at diakinesis, confirming DAVIE's count.

Thus *Anoda cristata* is a mixed species, having strains with haploid

numbers 15, and 18 respectively. This unusual condition will be referred to below. No phenotypic differences were observed.



FIGS 16-20 *Anoda* spp FIG 16 *A. cristata* (strain 1), metaphase I, side view FIG 17 *A. cristata* (strain 1), metaphase I, polar view FIG 18 *A. cristata* (strain 1), metaphase II, polar view showing median non-staining regions. FIG 19 *A. cristata* (strain 2), metaphase, root tip mitosis FIG 20 *A. cristata* (strain 2), metaphase I, polar view FIG 21 *Urena lobata*, metaphase, root tip mitosis FIGS 16, 17 and 18 $\times 3100$ FIGS 19, 20 and 21 $\times 4700$.

CHROMOSOME NUMBERS IN THE MALVEAE

In most large genera of the tribe Malveae (*Malva*, *Sida*, *Abutilon*, *Lavatera*, *Althaea*) the great majority of the species have chromosome numbers which lie in an euploid series with haploid numbers 7, 14, 21, 28, 35, 42, 56 (see especially SKOVSTED 1935, DAVIE 1933 and 1935). The evidence for the base number 7 is incontrovertible in these genera, in spite of the existence of a few possible aneuploid species in *Lavatera*, of one in *Sida*, and of two species with $n = 8$ in *Abutilon*,

for these aberrant numbers may easily have been derived from a 7 series. On the other hand, the species included in the sub-genus *Eusphaeralcea* of *Sphaeralcea* have haploid numbers 5, 10, 15 and 25. Here it is equally clear that the base number is 5 (WEBBER 1936).

Table 2 and diagram I summarise all the chromosome counts in species of the Malveae published to date and include those recorded here for the first time. They demonstrate clearly the predominance of chromosome numbers which are multiples of 7 or of 5. Hence the basic number of the tribe is very probably one of these two numbers. Which is more likely? Fifty-four of the species and varieties included in Table 2, representing eight of the thirteen genera studied, have chromosome numbers falling in the 7 series. In the 5 series occur 26 species and forms of *Eusphaeralcea*, 4 species of *Sidalcea*, 2 of *Malvastrum*, and 3 of *Anoda*. Thus it is evident that the 7 series is far more widely distributed in the tribe than the 5 series, which is almost confined to *Sphaeralcea*. (The fact that some of the counts of higher multiples of 7 are uncertain is not of importance).

TABLE 2. — DISTRIBUTION OF CHROMOSOME NUMBERS IN THE MALVEAE

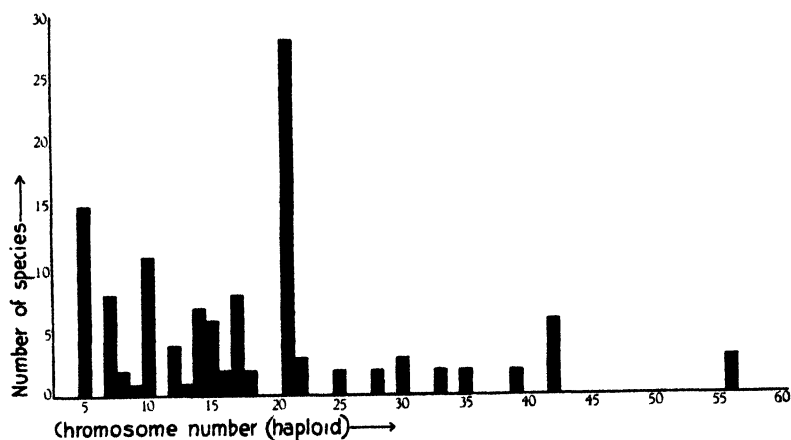
Chromosome number	Number of species	Chromosome number	Number of species
5	15	18	2
7	8	21	28
9	2	22	3
9	1	25	1
10	11	28	1
12	4	30	2
13	1	33	1
14	7	35	1
15	6	39?	1
16	2	42	6
17	8	56	3

This evidence is, in my opinion, sufficient to justify the conclusion that $b = 7$ (GATES 1935) for the Malveae, in which case the number

5 in *Eusphaeralcea* or its ancestors must have been derived from 7. The alternative hypothesis that both 5 and 7 are basic demands, of course, a polyphyletic origin of the tribe.

In conclusion it may be noted that the majority of species in the 7-series are hexaploids. It is decidedly unusual to find high polyploids so obviously favoured by conditions of survival

DIAGRAM I. DISTRIBUTION OF CHROMOSOME NUMBERS IN THE MALVEAE.



c) Tribe *Urcneae*

U r e n a

Twenty-eight chromosomes were counted at mitotic metaphase in root tips of *U. lobata* fixed in Navashin, thus confirming SKOVSTED's count. Two pairs with satellites were clearly visible in my preparation (fig. 21) but are not shown in SKOVSTED's figure.

P a v o n i a

Somatic counts in root tips of *Pavonia fruticosa* showed that 42 was the diploid number in this species.

CHROMOSOME NUMBERS IN THE URENEAE

As yet, the chromosome numbers of few species of this tribe have been recorded. All those known, however, are multiples of 7 and so the conclusion may be drawn that here also $b = 7$. As in the Malveae, many of the species are high polyploids. The distribution of chromosome numbers known at present is given in Table 3.

TABLE 3. — DISTRIBUTION OF CHROMOSOME NUMBERS IN THE URENEAE

Chromosome number	Number of species
14	5
21	2
28	3
42	1
56	2

d) Tribe *Hibisceae**Hibiscus*

The small size and large number of the chromosomes in this genus make counting extremely difficult. For this reason the number of chromosomes in several species can only be stated approximately.

Counts at mitotic metaphase in root tips have been made in seven species as follows: *H. angulosus* $2n = 56$ (fig. 22); *H. cannabinus* $2n = c. 72$, *H. collinus* $2n = 42$ (fig. 23); *H. costatus* $2n = 36$, *H. esculentus* (Strain 1) $2n = c. 66$, *H. peduncularis* $2n = c. 72$, *H. Trionum* $2n = 28$ (fig. 24).

Counts at stages of pollen mother cell meiosis gave the following numbers: *H. esculentus* (Strain 2) $n = c. 66$, *H. diversifolius* $n = c. 72$, *H. vitifolius* $n = 33$ (fig. 25).

Thespesia

Several counts at mitotic metaphase in the two species *T. lampas* and *T. populnea* showed that $2n = 26$ in each case. A somatic plate of *T. lampas* is illustrated in Fig. 26. The chromosomes in this plate ranged from 3.0μ to 1.7μ long. Several clear cases of attachment of chromosomes to the nucleolus were observed in prophase nuclei of the same species.

Gossypium

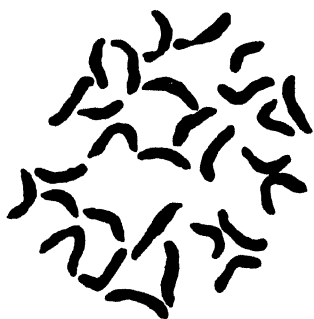
Root tip counts in two wild American species, *G. Armourianum* KEARNEY and *G. Harknessii* BRANDEG., confirmed the earlier counts of $n = 13$ in each case made by WEBBER (1934).



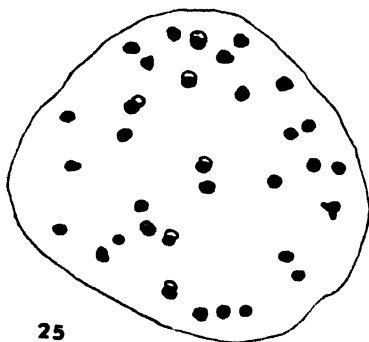
22



23



24



25



26

FIGS 22-25. *Hibiscus* spp. FIG 22. *H. angulosus*, metaphase, root tip mitosis. FIG. 23. *H. collinus*, metaphase, root tip mitosis. FIG. 24. *H. Trionum*, metaphase, root tip mitosis. FIG. 25. *H. vitifolius*, metaphase I. The line represents the inner limit of the perinuclear zone. FIG. 26. *Thespesia lampas*, metaphase, root tip mitosis. FIG. 22 $\times 3100$. Remainder $\times 4700$.

CHROMOSOME NUMBERS IN THE HIBISCEAE

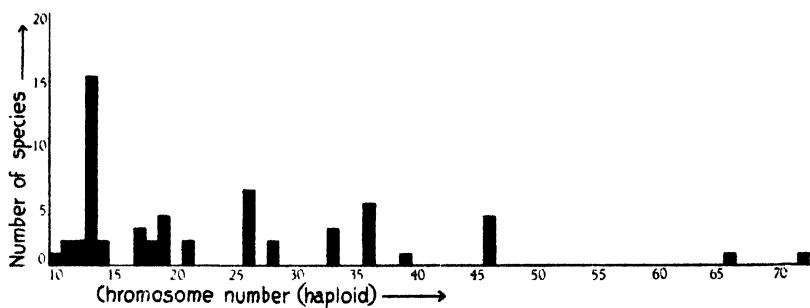
The distribution of chromosome numbers in the *Hibisceae* is given in Table 4 and expressed graphically in diagram II. In *Gossypium* and certain other genera (*Thespesia*, *Cienfuegosia*, *Shantzia*) the apparently secondarily balanced number 13 has been established, and so, owing to the disproportionately large number of *Gossypium* species which have been examined, there is a marked peak at $n = 13$ in diagram II. Apart from this peak there is no feature in diagram II which suggests a possible basic number of the tribe. Hence other and less satisfactory evidence must be used in the attempt to deduce this number. DAVIE (1933) concluded that the cottons and their allies had been derived from ancestors with $n = 7$, by doubling of the chromosome number followed by fusion of two chromosomes into one. He based his view on this evidence: (1) secondary association occurs in *Gossypium herbaceum* (LAWRENCE 1931) with a maximum association of 6 groups of 2 and 1 single bivalent (DAVIE 1933); (2) one pair of chromosomes at mitosis (DAVIE 1933) and one bivalent at meiosis (DENHAM 1924a) are considerably larger than the remainder of the complement; (3) the basic number in the related Malveae is probably 7, (DAVIE 1933). On the other hand, SKOVSTED (1937) concluded that 6 was the ancestral chromosome number of the cottons on the evi-

TABLE 4. — DISTRIBUTION OF CHROMOSOME NUMBERS IN THE HIBISCEAE

Chromosome number	Number of species	Chromosome number	Number of species
10	1	26	6
11	2	28	2
12	2	33	3
13	15	36	5
14	2	39	1
17	3	c. 46	4
18	2	c. 66	1
19	4	c. 72	1
21	2		

dence: (1) The maximum secondary association in *Gossypium aridum* is five groups of 2 and one group of 3 bivalents (SKOVSTED 1937). (2) The haploid chromosome number in species of the nearly related genera *Gossypoides* and *Kokia* is 12 (SKOVSTED 1937). In view of the fact that the association observed may be due in whole or in part to segmental homology (see CATCHESIDE 1934, ALAM 1936) and not to

DIAGRAM II. DISTRIBUTION OF CHROMOSOME NUMBERS IN THE HIBISCEAE



complete ancestral homology between bivalents, secondary association is only of value as corroborative evidence and should not be used as the „corner stone” of a hypothesis. Also, the correctness of the observation by DAVIE and DENHAM of a pair of chromosomes larger than the remainder of the complement is open to doubt in view of the measurements given by SKOVSTED (1934) and ARUTJUNOVA (1936). Hence it may be concluded that there is at present insufficient evidence to justify the adoption of either of the above numbers, 6 or 7, as the basic number of the Hibisceae. The question, for the present, must remain open.

NOTE ON PROCHROMOSOMES

The presence of prochromosomes is apparently a characteristic of the family. They always show clearly in material fixed in Navashin but are sometimes not apparent in osmic fixed material. They stain well in resting nuclei of root tips and anther wall cells crushed in aceto-carmine. Counts of prochromosomes have been made in tetrad nuclei of *Anoda cristata* and in root tip nuclei of *Gossypium Armourianum*. The counts agreed with haploid and diploid numbers of these species respectively ($n = 15$, and $2n = 26$).

DISCUSSION

Several examples of intraspecific variation in chromosome number occur in the Malvaceae. Such variation may be of two kinds, the first in which the chromosome numbers of the different strains are all simple multiples of a single basic number, and the second in which the one strain has perhaps 1, 2 or 3 more pairs of chromosomes than the other. These two kinds of variation may be called euploid and aneuploid variation respectively.

It is now evident that intraspecific tetraploidy is widespread in the genus *Hibiscus*. SKOVSTED's list (1935) includes a single species, *H. esculentus*, with diploid and tetraploid races. Recently NAKAJIMA (1936) has reported a race of *H. Trionum* with $2n = 28$, whereas previous counts by SKOVSTED and DAVIE had $2n = 56$. This then, is apparently a second species with diploid and tetraploid races. My own determinations confirm the conclusion with regard to the above two species and indicate that three more species, *H. cannabinus*, *H. collinus* and *H. vitifolius* may have diploid and tetraploid races also. In *H. cannabinus* my count is $2n = 72$, whereas SKOVSTED (1935) and BRESLAVETZ *et al.* (1934) have $2n = 36$. In *H. collinus* I find $2n = 42$, compared with SKOVSTED's count of $2n = c. 92$. This may well mean that $2n = 84$ really, in which case his race would be tetraploid relative to mine. Finally, I counted 33 bivalents at metaphase I in *H. vitifolius* compared with SKOVSTED's count of $2n = 34$. A possible explanation of this discrepancy is that I may have examined an aneuploid segregate from a relatively tetraploid race. That such aneuploid variation does occur in high polyploids is demonstrated by MUNTZING's (1935) observations on a race of the synthesized *Nicotiana digluta* which had 68 chromosomes instead of 72 as in the original line. The comparison is not strict, for *Nicotiana digluta* is an allopolyploid while the *H. vitifolius* race is presumably derived from an autotetraploid strain. The origin and behaviour of tetraploid races in nature and especially in cultivation is now so well known that discussion is rendered unnecessary.

In addition to *Hibiscus*, no less than eight species of the sub-genus *Eusphaeralcea* have polyploid races. The extreme condition is represented by *S. ambigua* and *S. Emoryi* with haploid numbers of 5, 10, and 15 in the one species and 10, 15, and 25 in the other (WEBBER

1936). These two cases may be likened to BLACKBURN's (1933) example of *Silene ciliata* in which diploid, tetraploid and 16-ploid races exist. The euploid races of *Eusphaeralcea* are presumably autopolyploid, for had hybridization been involved in their ancestry greater taxonomic differences would be expected between races referred to a single species. It follows that the races of *S. Emoryi* with $n = 10$, $n = 15$ and $n = 25$ are probably autopolyploid, in which case it must be inferred that a further race with $n = 5$ exists, or existed formerly, for it is impossible for autopolyploids with $n = 15$ and $n = 25$ to arise from a species in which the haploid number is 10. The remaining euploid variation which occurs in the sub-genus could easily be accounted for on an autopolyploid hypothesis.

In high autopolyploid strains such as the 16-ploid *Silene ciliata* of BLACKBURN and the race of *S. Emoryi* with $n = 25$ (presumably decaploid) to which reference has just been made, a high degree of multivalent formation would be expected, and, as a consequence of irregular segregation, frequent aneuploid individuals should occur. This, in turn, should lead to a high degree of polymorphism within the species. Multivalents were observed in the 16-ploid *Silene* by BLACKBURN (1933), but in the absence of more information further speculation is unwarranted.

It will be recalled that aneuploid variation occurs in *Anoda cristata* and *Malope trifida*, these two species having races with haploid numbers 15 and 18, and 22 and 25 respectively. Three hypotheses may be advanced to explain such variation in chromosome number.

- 1) The extra chromosomes in the race with the higher chromosome number may be inert as in Maize (RANDOLPH 1928). This hypothesis cannot be disregarded until crosses are made and the progeny examined for variation.

- 2) A change in the number of centromeres may have occurred accompanied merely by „qualitative” structural changes. (That is, changes not including duplication or deficiency). (NAVASHIN 1932). Such changes would not alter the gene balance and therefore, apart from possible position effect, should not be detectable phenotypically.

- 3) Finally, secondary polyploidy might be invoked as an explanation. Though here we encounter the fact that the two lines are identical morphologically and one would scarcely expect to find that

a plant with the constitution of a threefold tetrasomic was indistinguishable from the parent line.

In the course of evolution (2) probably never occurs as suggested. At present it seems that a greater or less degree of duplication or deficiency must be involved, especially of the regions adjoining the centromeres.

The study of F_1 hybrids between races differing in chromosome number, such as those referred to above, should give results of considerable interest.

SUMMARY

1. Chromosome numbers of 32 species from 13 genera representing three of the four tribes of the Malvaceae are reported. They are summarized in Table 1 (p. 433).

2. It is possible to identify certain of the chromosomes of the diploid species of *Abutilon* by characteristic features of their morphology.

3. Attachment of chromosomes to the nucleolus in the prophase of mitosis or meiosis has been observed in several genera, especially *Abutilon* and *Sidalcea*.

4. Secondary association probably occurs in most polyploid species examined, but is not pronounced.

5. Tetraploid strains occur in several *Hibiscus* species.

6. Of three races of *Anoda cristata* L., two had the haploid number 15, while the third had $n = 18$. The problem of the origin of such variant strains is discussed.

7. It is concluded that 7 is the basic number in the *Malveae* and *Ureneae*, and probably also in the *Malopeae*. The basic number in the *Hibisceae* is as yet uncertain, though the evidence is not incompatible with $b = 7$ as in the *Malveae* and *Ureneae*.

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ZWEI VERSCHIEDENE FÄLLE SOMATISCHER SPALTUNG IN DER BLÜTENEPIDERMIS HETEROZYGOTER PFLANZEN

von

R. PRAKKEN

Laboratorium voor Erfelykheidsleer, Wageningen
(Manuskript eingegangen am 24 November 1937)

A. NICOTIANA

Im Jahre 1933 wurden vom Herrn DOJES *Nicotiana atropurpurea grandiflora* (mit dunkelroten Blüten) und *Nicotiana Tabacum* (Amersfoorter Rasse, mit rosa Blüten) gekreuzt. Die F₁ wurde (1934) angebaut und beschrieben vom Herrn G. J. DE GROOT. Alle F₁ Pflanzen waren rotblütig, nur eine Spur heller als die *atropurpurea* Rasse : rot ist fast völlig dominant über rosa. Die Farbe ist lokalisiert in den Epidermiszellen. Eine der rotblütigen F₁ Pflanzen nun zeigte einen Ast mit rosa Blüten wie die Amersfoorter Rasse. Dieser Ast und auch ein normal rotblühender derselben F₁ Pflanze wurden geselbstet. Die Analyse der F₂ wurde (1935) ausgeführt vom Herrn F. OPPENOORTH. Das Resultat war eine monofaktorielle Spaltung in den beiden F₂ Familien:

	gefunden			erwartet 3 : 1		D/m
	rot	rosa	total	rot	rosa	
roter Ast . .	229	67	296	222	74	0,94
rosa Ast . .	74	24	98	73.5	24.5	0.12

Die tieferen (subepidermalen) Schichten am rosa Ast waren also unverändert (d.h. heterozygot) geblieben. Es sei hier hingewiesen auf eine Mitteilung von CLAUSEN und GOODSPEED (1923) über einen ganz

ähnlichen Fall bei *Nicotiana*, wahrscheinlich dasselbe Faktorenpaar ($P - p$, carmine-pink) betreffend; auch da zeigten die Nachkommen-schaften von beiderlei Ästen dieselbe Spaltung. Ausserdem wurden von ihnen vom rosa Ast Stecklinge erhalten, alle mit rosa Blüten. Dann aber lieferten diese rosablühenden Pflanzen auch Wurzelstecklinge, welche ohne Ausnahme rotblütig waren: die tieferen Schichten waren also von der Veränderung in der Epidermis nicht betroffen worden.

B. PHASEOLUS

Für die Blütenfarbe von *Phaseolus* ist eine triple allelomorphe Serie bekannt: vv Blüten sind weiss, $v_{lae}v_{lae}$ Blüten hell laeliafarbig

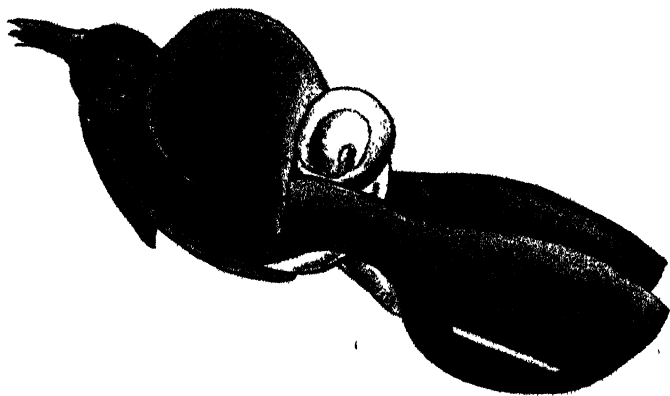


ABB. 1. *Phaseolus vulgaris*; somatische Spaltung in einer heterozygoten Vv_{lae} Blüte: gepaarte VV und $v_{lae}v_{lae}$ Streifen.

und VV Blüten violett (LAMPRECHT, 1936, p. 151); auch Stengel- und Samenhautfarbe werden von diesen Faktoren beeinflusst. Heterozygote Vv_{lae} Pflanzen zeigen deutlich etwas heller violette Blüten als VV Pflanzen (R. PRAKKEN, 1934, p. 184; das damals v genannte Allel wird jetzt mit $v_{lae} v_{lae}$ angedeutet). In diesem Sommer (1937) nun fand ich auf einer hellvioletten Vv_{lae} Pflanze eine abweichende Blüte (s. Abb. 1). Eine der im ganzen hellvioletten Flügel zeigte auf der Auzsenseite zwei nebeneinanderliegende Streifen von abweichender Farbe ("twin spots"): der eine violett, der andere

sehr hell lacliafarbig. Also in einer Vv_{lae} Blüte nebeneinander Streifen mit genau die VV und die $v_{lae}v_{lae}$ Farbe. Die Breite des dunklen Streifen war ungefähr zweimal die des hellen; die Länge war nur sehr wenig grösser. Wie bei *Nicotiana* ist die Farbe in den Epidermiszellen der Blüte lokalisiert; sie ist beim *Phaseolus*-Flügel am stärksten in der Auszenepidermis.

Die Ursache des Auftretens rezessiver Merkmale auf heterozygoten Individuen kann eine Mutation sein. Meistens aber hat man Elimination eines Chromosoms (mit dem dominanten Faktor) angenommen (EMERSON 1924, BRIDGES 1925, SEREBROVSKY 1925). Auch das Verschwinden von einem Teile eines Chromosoms ist angenommen worden (HERTWIG und RITTERSHAUS 1929, PATTERSON 1930). Dasz es sich vielleicht im Allgemeinen handelt um eine Chromosomen-Abnormalität und nicht um eine Faktor-Mutation, ist schon darum wahrscheinlich, dasz manchmal zwei oder mehr gekoppelte dominante Faktoren zugleich verschwinden (EMERSON 1924, FROST 1921, 1924) und dasz oft die Änderungen der Farbe von Wachstumsänderungen begleitet werden (JONES, 1936a). Das Auftreten von gepaarten Flecken (beim *Zea Mais* Aleuron studiert von JONES, 1936a und b) kann weder mit der Annahme einer Faktor-Mutation noch mit der blossen Elimination eines Chromosoms erklärt werden. Wahrscheinlich entstehen bei einer bestimmten Zellteilung zwei korrelativ veränderte Zellen. Wenn es sich dabei um ganze Chromosomen handeln sollte bestehen wenigstens theoretisch verschiedene Möglichkeiten

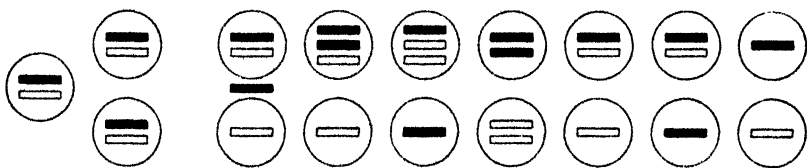


ABB. 2. Unregelmäßigkeiten ganzer Chromosomen als Ursache somatischer Spaltung (Verlust, Nichttrennen, Nichtteilen).

von Elimination, Nichtteilen und Nichttrennen für die Entstehung von gepaarten oder ungepaarten Flecken (Abb. 2).

Nun hat aber STERN (1936) in einer ausführlichen Arbeit über "Somatic crossing-over and segregation in *Drosophila melanogaster*" es sehr wahrscheinlich gemacht, dasz (wenigstens bei *Drosophila*) das

Auftreten von rezessiven Flecken an heterozygoten Individuen nicht verursacht wird durch Verlust, Nichttrennen oder Nichtteilen von ganzen Chromosomen, sondern die Folge somatischer Überkreuzung ist. Die Überkreuzung findet statt, wenn die beiden homologen Chromosomen schon der Länge nach gespalten sind, zwischen zwei von den vier Chromatiden. Für die Spindelansatzstellen ist die Teilung immer äquationell. Wie man aus der Abb. 3, ersieht ist somatische Spaltung die Folge. Auch bei völliger Dominanz können gepaarte Flecken auftreten ("twin spots"), dann aber Flecken zweier verschiedenen rezessiven Faktoren.

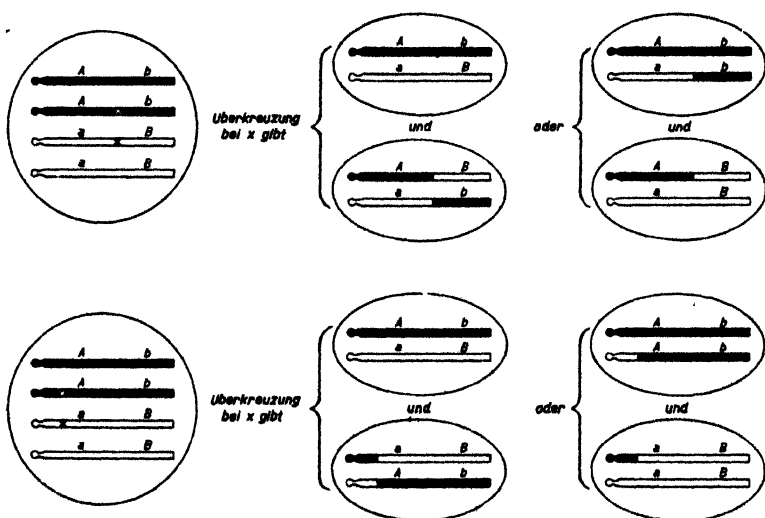


ABB 3. Überkreuzung auf dem Viererstrangstadium als Ursache somatischer Spaltung (nach STERN; etwas verändert); oben: *bb* Fleck; unten: gepaarte Flecken: *aa* und *bb*.

Die beiden obenbeschriebenen Fälle somatischer Spaltung können also folgendermassen verursacht werden: bei *Nicotiana* durch Mutation, durch Ausfall von einem Teile eines Chromosoms oder eines ganzen Chromosoms, durch Unregelmäßigkeiten in der Verteilung ganzer Chromosomen oder durch somatische Überkreuzung; bei *Phaseolus* durch solche Unregelmäßigkeiten in der Verteilung ganzer Chromosomen wobei zwei korrelativ veränderte Zellen entstehen oder durch Überkreuzung.

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STUDIES ON THE GENETICS AND CYTOLOGY OF
DROSOPHILA ANANASSAE

by

HIDEO KIKKAWA

Zoological Institute Kyoto Imperial University

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With 7 plates

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INTRODUCTION

Since the end of 1933, I have devoted myself to genetical and cytological studies of *D. ananassae*. This species was originally selected as material because of its excellent viability and high mutability. With the progress of the work, however, it has become clear that this species has some peculiarities in its genetical and cytological behaviors, which are probably unique among all the *Drosophila* species thus far investigated.

Although my investigations on these points have not yet come quite to the conclusive point of main problems, still it seems worth while to publish the results of my investigations so far obtained. This paper, therefore, is intended primarily to bring together the data and to indicate the general trend of the results.

Before going further, it is a pleasure to record here my gratitude to Professor T. KOMAI for his kind guidance and valuable aid in many ways. I am much indebted to Professors D. MORIWAKI and A. H. STURTEVANT for their kindness in placing their precious stocks at my disposal and in giving me valuable information. My sincere thanks are also due to the members of our laboratory who have cooperated with me in the work — Dr. M. CHINO, Mr. S. FUJII and others. Further, I wish to express my hearty thanks to the following persons whose skill has produced the figures for this paper — Messrs. Y. MAKINO (Figures 1, 6, Plates VI, VII) and K. SHIMADA (Figure 5). I also convey my thanks to the Hattori Hôkôkwai for the financial aid given to the work carried out in the laboratory.

REVIEW OF LITERATURE

The first mutant discovered in the species (*D. caribbea*, of STURTEVANT) was an autosomal recessive gene, curved wing, which was reported by STURTEVANT in 1921 (Carnegie Inst. Wash. 301, p. 13). Previous to this, METZ (1916) had already described the chromosome type of this species as L type. Work on *D. ananassae* had since then been suspended.

Recently, however, several persons began independently to study the genetics and cytology of this species. Thus, MORIWAKI (1934, 1935*abd*, 1936*abc*, 1937*ab*) published a number of papers on genetical

behaviors of some mutant characters and on the male crossing over. KAUFMANN (1936^{ab}, 1937^{ab}) has shown many important results concerning chromosome behavior. Recently, MONOD and POULSON (1937) have reported an interesting phenomenon as to interspecific transplantation among *melanogaster* groups including *ananassae*. I also published several papers (KIKKAWA, 1936^a, 1935^a, 1937^{ab}) regarding some cytological and genetical phenomena. Further, in the series of *Drosophila* Information Service, reports are given by several investigators (No. 2, pp. 53-54; No. 3, p. 46; No. 4, pp. 56-58, 61; No. 5, p. 23, 25; No. 7, pp. 79-84).

PART I. GENERAL PROBLEMS

I. MATERIALS

The specific name *Drosophila ananassae* was first used by DOLESCHALL in 1858. The taxonomical descriptions of this species involving the synonyms are given by the following authors:

D. ananassae: DOLESCHALL (1858) Nat. Tijds. Ned. Ind. 17; 128, 89 (I have not read the original paper). DE MEIJERE (1908) Tijds. v. Ent. 51; 159. DUDA (1924) Arch. f. Naturgesch. 90; 214 (1925) Ibid. 91; 211-213. (1926) Supplementa Entomologica 14; 98. PENG (1937) Annot. Zool. Japon. 16; 26-27.

D. caribbea: STURIEVANT (1916) Ann. Ent. Soc. Amer. 9; 335. (1921) Carnegie Inst. Wash. 301; 92-93.

D. errans? MALLOCH (1934) British Mus. (Nat. Hist.) Part 6, 300-301.

D. imparata: WALKER (1859) Proc. Linn. Soc. 3; 126, 164 (I have not read the original paper).

D. similis? LAMB (1914) Trans. Linn. Soc. 16; 347.

D. ananassae agrees fairly closely in its general structural features with *D. melanogaster*. However, there are many slight differences; e.g. the eye is considerably larger, the body color more dull-yellowish and the costal index of the wing is much smaller than in *D. melanogaster*. Especially noticeable is the absence of a distinct sex-comb that is present in the male of *D. melanogaster*, though there are clusters of hairs on the first and second tarsal joints of the prothoracic leg of the male (Fig. 1).

There are also some physiological differences between the two species. In the normal condition (about 23°–27° C), the development rate of the two species is nearly the same, while at low temperature (about 10° C), the rate of development of *ananassae* becomes lower than that of *melanogaster*. The fact that *D. ananassae* is sensitive to cold conforms well with its geographic range of distribution. Namely, it is found only in tropical or subtropical regions; while *D. melanogaster* is distributed all over the world except in the arctic region.

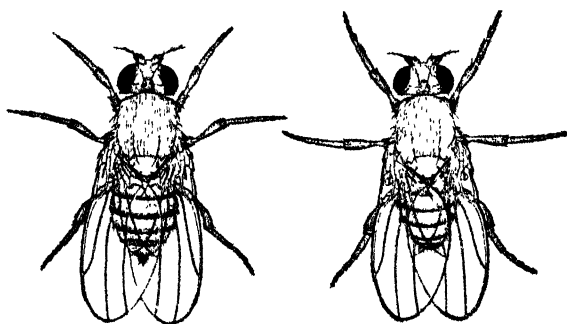


FIG. 1 Wild type of *D. ananassae*. Left: female
Right: male.

In nature, *D. ananassae* seems to gather and breed on decaying fruits; but according to STURTEVANT (1918), it frequents human excrement, and sometimes breeds on it.

As far as I know, *D. ananassae* resembles *D. bipectinata* DUDA most closely. At a glance, they are almost indistinguishable; the genital apparatus, the germinal chromosomes and even the salivary chromosomes, especially the landmarks of proximal and distal parts of the right arm of the X-chromosome, are very similar. The only marked difference between the two species is found in the presence in *D. bipectinata*, and the absence in *D. ananassae*, of two distinct sex-combs in the male.

When the different sexes of the two species are placed in one culture bottle, they try to copulate with each other, though the attempt is always unsuccessful. Such behavior is never observed when *ananassae* is placed with species other than *bipectinata*.

My own records of collection of *D. ananassae*, as well as those found

in literature, show clearly that this species lives in tropical or sub-tropical regions. It is widely distributed and common in southern states of North America, Central America, South America, Samoa, South Sea Islands, Ryukyu, Formosa and in the southern districts of China (Table 19).

II. CHROMOSOME OF *D. ananassae*

A. Chromosomes in germ cells

The female-chromosome group of *D. ananassae*, as stated by METZ (1916), consists of four pairs of the V-shaped chromosomes, one of which is considerably smaller than the others (Fig. 2 *a, b*). In the spermatogonial metaphase, one of the large V-shaped chromosomes is replaced by a small J-shaped chromosome, and accordingly, these heteromorphic chromosomes are regarded as representing the sex-chromosomes, i.e. X and Y (Fig. 2 *c*).

It is interesting that the form of Y-chromosome seems to differ according to the strain. In his earlier paper, METZ (1916) reported that the material obtained from Panama and Cuba had a rod-shaped Y-chromosome. Recent works, however, reveal that all of the specimens examined possess a J-shaped Y-chromosome (KAUFFMANN, 1936*a*, the Tuscaloosa strain and a Japanese strain; KIKKAWA, four Japanese strains and one Chinese strain). Such a racial difference in the form of the Y-chromosome reminds us of the similar case observed in *D. pseudoobscura* (DOBZHANSKY, 1935, 1937).

One arm of a longer autosome, probably the left arm of the third chromosome, shows a pronounced sub-median constriction as pointed out by KAUFFMANN (1936*ab*, 1937*b*) (Fig. 2 *f, i, m, p*; Pl. V *b, c*). Schematic figures of germinal chromosomes are given in Figs. 3 and 5.

B. Chromosomes in nerve cells

In the aceto-carmine preparations, the structure of the metaphase chromosomes in a large nerve cell is generally more distinct than that in a germ cell. In good preparations, constrictions of each chromosome are clearly observable. Several figures of the neurocyte chromosomes are shown in Fig. 2 *m, n, o, p*. The cytological behavior of the

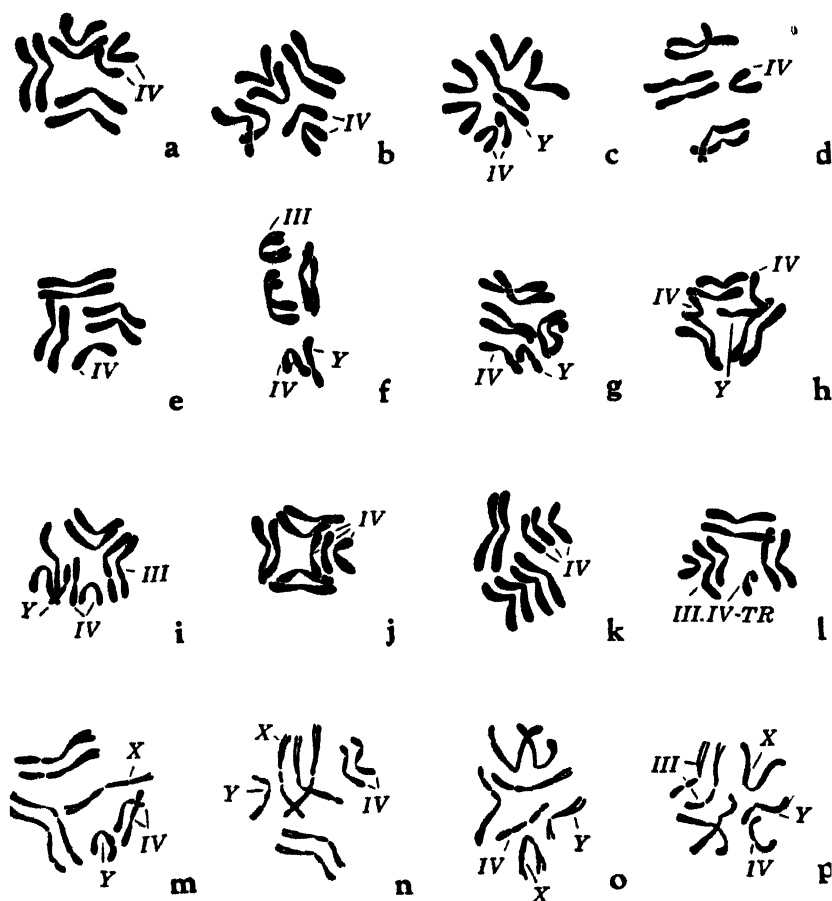


FIG. 2. Chromosomes of *D. ananassae*. *a*, oögonial metaphase of normal type. *b*, the same. *c*, spermatogonial metaphase of normal type. *d*, oögonial metaphase of XX haplo-IV type (Pl. V *a*). *e*, the same. *f*, oögonial metaphase of XXXY haplo-IV type (Pl. V *b*). *g*, the same. *h*, oögonial metaphase of XXXY (Pl. V *c*). *i*, the same (Pl. V *c*). *j*, oögonial metaphase of triplo-IV (Pl. V *d*). *k*, the same. *l*, oögonial metaphase of III-IV translocation (*Plum*). *m*, metaphase chromosomes in a neurocyte of normal male *n*, the same *o*, metaphase chromosomes in a neurocyte or XY haplo-IV male. *p*, the same. (Acetocarmine preparations. \times ca. 3000).

TABLE 1

Mutant characters of *D. ananassae*. They are arranged in order: full name, symbol, locus, first discoverer, date of discovery and main character affected. In the column "date of discovery" the symbols are used according to the by-laws of the DIS, for example, 33a2 indicates 1933, January, 2nd, and 35d11, 1935, April 11th. These mutants are described briefly in Appendix I.

THE FIRST CHROMOSOME

Name	Symbol	Locus	Discoverer	Date of discovery	Main character affected
dwarf	<i>dp</i>	0.0	KIKKAWA	35i28	Various parts
Notch	<i>N</i>	15.5	Do.	34d21	Wing margin
white	<i>w</i>	16.7	MORIWAKI	33j19	Eye color
apricot	<i>w^a</i>	16.7	KIKKAWA	35h19	Eye color
Minute-Ia	<i>M-Ia</i>	30.5	Do.	36 17	Bristles
garnet	<i>g</i>	37.5	Do	34g4	Eye color
garnet ²	<i>g²</i>	37.5	Do.	34i29	Eye color
forked	<i>f</i>	41.5	Do.	34i16	Bristles
forked ²	<i>f²</i>	41.5	Do.	35e22	Bristles
vermillion	<i>v</i>	44.0	Do.	34k10	Eye color
miniature	<i>m</i>	45.5	Do.	33j15	Wings
dusky	<i>dy</i>	46.2±	Do.	36k17	Wings
purplish	<i>ph</i>	65.0	MORIWAKI	35d23	Eye color
broad	<i>br</i>	72.0	KIKKAWA	35d9	Wings
scute	<i>sc</i>	96.0	MORIWAKI	35g7	Bristles
yellow	<i>y</i>	96.2	Do.	36b21	Body color
achaete	<i>ac</i>	102.0	Do.	36b13	Bristles
Interrupted	<i>Ir</i>	109.6±	Do.	32i28	Veins
singed	<i>sn</i>	112.7	KIKKAWA	34j10	Bristles
singed ²	<i>sn²</i>	112.7	Do.	35k11	Bristles
cut	<i>ct</i>	117.6	Do.	33k1	Wing margin
cut ²	<i>ct²</i>	117.6	Do.	35k29	Wing margin
crumploid	<i>cl</i>	117.6±	MORIWAKI	35d5	Wings
abnormal	<i>ab</i>	163.6	KIKKAWA	35f20	Genitalia

THE SECOND CHROMOSOME

Name	Symbol	Locus	Discoverer	Date of discovery	Main character affected
crooked	<i>ck</i>	?	MORIWAKI	33f26	Legs
lethal-1	<i>l-1</i>	?	Do.	34d?	Viability
lethal-3	<i>l-3</i>	?	Do.	?	Viability
cardinal	<i>cd</i>	0.0	KIKKAWA	34b24	Eye color
curved-IIa	<i>c-IIa</i>	1.0?	Do.	34c10	Wings
spread	<i>sd</i>	20.3±	Do.	36a11	Wings
Plexate	<i>Pt</i>	23.6	MORIWAKI	33k15	Veins
Plexate ²	<i>Pt²</i>	23.6	KIKKAWA	34i10	Veins
ski	<i>sk</i>	23.6±	MORIWAKI	33f26	Wings
spineless	<i>ss</i>	24.5	KIKKAWA	35f10	Bristles
Off	<i>Off</i>	37.8	MORIWAKI	36b22	Bristles
tiny bristle	<i>tb</i>	39.5	KIKKAWA	34h15	Bristles
ebony	<i>e</i>	49.1	Do.	36i8	Body color
missing	<i>ms</i>	56.7±	MORIWAKI	35k13	Bristles
prickly	<i>pk</i>	60.6	KIKKAWA	36k9	Bristles
slender	<i>sl</i>	66.0±	Do.	34h1	Bristles
Minute-IIa	<i>M-IIa</i>	74.0?	Do.	34f25	Bristles
retracted	<i>rt</i>	?	MORIWAKI	33l26	Wings
lethal-2	<i>l-2</i>	?	Do.	34e?	Viability
lance	<i>la</i>	?	KIKKAWA	34j11	Wings
stubble	<i>sb</i>	?	Do.	35b19	Bristles
obliterated	<i>ob</i>	?	Do.	35c4	Veins
Minute-IIc	<i>M-IIc</i>	?	Do.	35i30	Bristles
extended	<i>ex</i>	?	MORIWAKI	36a22	Wings
Minute-IIb	<i>M-IIb</i>	?	Do.	35h8	Bristles
curved-IIb	<i>c-IIb</i>	?	KIKKAWA	36l12	Wings

THE THIRD CHROMOSOME

Name	Symbol	Locus	Discoverer	Date of discovery	Main character affected
ski-III	<i>sk-III</i>	0.0	MORIWAKI	3516	Wings
Minute-IIIa	<i>M-IIIa</i>	19.0±	KIKKAWA	34j24	Bristles
broken	<i>bn</i>	22.0±	Do.	35e31	Veins
Minute-IIIb	<i>M-IIIb</i>	26.5	Do.	34k19	Bristles
curved-IIIa	<i>c-IIIa</i>	27.5±	Do.	35i27	Wings
gap	<i>gp</i>	33.5±	Do.	35h22	Veins
balloon	<i>ba</i>	35.0±	MORIWAKI	33g8	Wings
Plum	<i>Pm</i>	35.0	KIKKAWA	35h22	Eye color
lethal-IIIa	<i>l-IIIa</i>	35.0±	Do.	36h	Viability
extra	<i>et</i>	36.5±	Do.	35k14	Wings and Veins
Barb	<i>Bb</i>	39.5	Do.	35a14	Bristles
plexus	<i>px</i>	83.5	Do.	34e8	Veins
lanceolate	<i>ll</i>	?	Do.	35b23	Wings
Minute-IIIc	<i>M-IIIc</i>	?	MORIWAKI	35l6	Bristles
Enhancer	<i>En</i>	?	KIKKAWA	36k	Crossover frequency

THE FOURTH CHROMOSOME

bobbed-IV	<i>bb-IV</i>	0.0	MORIWAKI	34k28	Bristles
bobbed ²	<i>bb²</i>	0.0	KIKKAWA	35k11	Bristles
Haplo-IV	<i>H-IV</i>	—	Do.	35j3	Various parts

OTHER AUTOSOMAL MUTANTS

curved wing	<i>c</i>	?	STURTE-VANT	?	Wings
incomplete crumpled	<i>ic</i>	?	MORIWAKI	32b16	Veins
	<i>cr</i>	?	Do.	32i30	Various parts

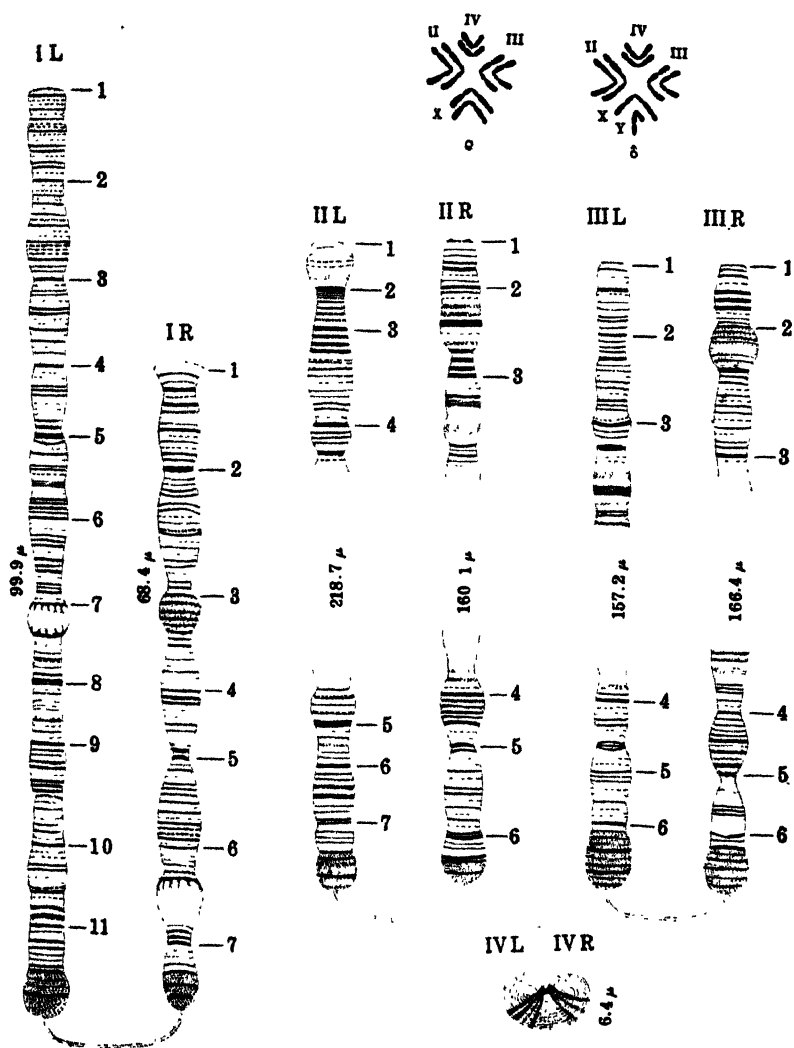


FIG. 3. Schematic figures of salivary gland chromosome of *D. ananassae*. Connection of the two arms of each chromosome with dotted line, is arbitrary. Right-upper figures show the metaphase chromosomes in a germ cell schematically.

prophase chromosomes in nerve cells has been studied by KAUFMANN (1936*ab*, 1937*ab*). His interesting result will be referred to in a later section.

C. Chromosomes in salivary gland cells

In the salivary gland nuclei of the female, as shown in my previous papers (1936*a*, 1937*a*) and in KAUFMANN's papers (1936*b*, 1937*ab*), only six chromosome strands radiate from the chromocenter instead of the eight which might be expected to result from conjugation of the four pairs of V-shaped homologues.

Two of the six strands are relatively short and represent the arms of the X-chromosomes. This identification may be easily made by comparison with salivary gland nuclei of male larvae, in which the X-chromosome exists in a slender haploid condition. The other four strands represent the arms of the second and the third chromosomes. Each strand has a characteristic arrangement of the chromatin bands and they can be easily distinguished from one another.

The shortest pair of autosomes are represented in the salivary gland cell by a small bipartite mass of heterochromatin material which forms a part of the chromocenter. In the most favorable preparations, however, there are occasionally observed a small number of chromatin bands in each arm of this pair.

Fortunately I have succeeded in discovering the relationship between the salivary chromosome strands and the genetic maps for the longer autosomes, by using an inversion which is located in the left arm of the second chromosome, and a reciprocal translocation involving the third and the fourth chromosome. The identification of the X-chromosome and fourth chromosome strands, has not yet been conclusively proved, but presumably the identification described here is correct at least as regards the X-chromosome.

In Fig. 3 the structure of the salivary gland chromosome for each strand is given rather schematically. It is hoped that these figures can be made more accurate through future investigation.

III. MUTANT CHARACTERS

In the following list, all mutant characters including multiple alleles are shown according to linkage groups (Table 1 See p. 464).

Besides these, data on the following mutants have been obtained through personal correspondence: *extended-b* (X, MORIWAKI), *cut*³ (X, Do.), *scute*² (X, Do.), *bent* (II, Do.), *missing-b* (II, Do.), *Beadex* (X, $w + 8.0 = 24.7$, SPENCER and STALKER; DIS. 7, p. 83) and *reduced* (II, STALKER; DIS. 7, p. 83).

Of the mutants given above, the following two seem worthy of description in some detail.

(1) *Plum* (III — 35.0): One male of purplish eye-color was found on August, 22, 1935, in a wild stock obtained from Isigakizima of the Ryukyu Group. Subsequent tests have proved that the eye-color mutant is dominant and mutable to normal, and further that it is associated with a reciprocal translocation between the third and fourth chromosomes. In the oögonial metaphase of a female involving this mutant in a heterozygous state, are found two abnormal chromosomes, i.e. a large and a small J-shaped chromosome (Fig. 2 i). These two abnormal chromosomes are to be regarded as representing the third and fourth chromosomes involving the translocation. Examination of the salivary gland chromosomes including *Plum*, have disclosed the nature of this chromosome aberration.

As shown in Part II, Chapter V, in the left arm of the third chromosome of this species, there are two different types, A and B, as to the arrangement of chromatin bands. The breakage is found in the B-type, at a point about one-third from the distal end (Pl. I a, d). Microscopic examination of both the germinal and salivary chromosomes, shows that the distal part of the third chromosome is attached with its breakage point to the fourth chromosome at a point close to the spindle-fiber attachment. On the other hand, the fragment of the fourth chromosome, i.e. the longer arm involving the *bobbed-IV* locus, is attached to the breakage point of the third chromosome with its proximal end, thus forming a mutual translocation. A schematic figure illustrating the above relation is shown in Fig. 4.

The *Plum* gene of *D. ananassae*, as in the similar gene of *D. melanogaster*, shows the following characteristics: (1) a dominant and eversporting eye-color, (2) accompaniment of a recessive lethal action (3) association with chromosome aberrations including a heterochromatic region (DUBININ and HEPTNER, 1935; GLASS, 1933; GOWEN and GAY, 1934; SCHULTZ and DOBZHANSKY, 1934; SCHULTZ, 1936; STERN, 1935; VAN ATTA, 1932, etc.). Moreover, *Plum* shows a

white eye-color in combination with the *vermilion* gene. Further details concerning this mutant will be given in a separate paper.

(2) *bobbed-IV* or *bobbed* (IV — 0.0): This mutant was first discovered by MORIWAKI on November 28, 1934, in a wild stock, and subsequently by the present author on November 11, 1936, in a wild stock obtained from Tainan in Formosa. As in other species of *Drosophila*, it is characterized in the female, by late emergence, short and slender bristles, and also often by scaly sclerites. The male, however, is generally normal as to these characters.

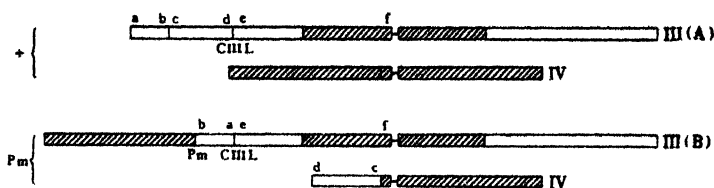


FIG. 4. Schematic figures showing the relation of the III-IV mutual translocation (*Plum*). The portion with oblique lines is assumed to be inert. CIII L shows the point in which a terminal inversion occurs (see Part II, Chapter V).

From the facts mentioned above, MORIWAKI (1935*d*) assumed that this mutant belongs to the X-chromosome as in other species. But, as he has stated in his recent papers (MORIWAKI, 1937*ab*), there are some peculiarities in its genetical behavior, which apparently contradict his previous assumption. My investigations on the genetics and cytology of this mutant, have revealed that it is located in the fourth chromosome.

Before presenting the linkage data of this mutant, it is necessary to state that the *bobbed* character of *ananassae* appears often in the male in some cultures. This unusual phenomenon apparently depends largely on the characteristic of the Y-chromosome in the stock used. Thus, when a *bobbed* female is mated to a male having Y with a weak dominant allele of *bb*, there are obtained several male offspring showing the *bobbed* character. In actual counting, however, it is difficult to separate such *bobbed* males from their normal brothers, because of the presence of intergrades. In Table 2 an example of such cases is represented.

TABLE 2

Variations of the bristle-length (right posterior dorsocentral) in the *bobbed* and the normal male. The length of the bristle obtained in this experiment was measured by the Sump method (see Appendix III).

Cross	<i>bb</i> ♀ × <i>bb</i> ♂	<i>bb</i> ♀ × + ♂
Length (× 145)	Number	Number
2.1—3.0	3	0
3.1—4.0	8	0
4.1—5.0	40	9
5.1—6.0	33	22
6.1—7.0	1	3
Total	85	34
Mean	4.79	5.33
Coefficient of Variance	14.59	8.87

As shown in this table, the average length of the bristles is nearly the same either in $IV^{bb}IV^{bb}Y^{+}$ or in $IV^{bb}IV^{+}Y^{+}$. But the coefficient of variance reaches 14.6 in the former case as compared with 8.9 in the latter, showing a wide deviation of the bristle-length in the former.

The first evidence that *bobbed* does not belong to the X-chromosome, was obtained by the following experiment. A virgin female with *vermilion* eye-color was mated to a male showing the *bobbed* character and the F_2 offspring were examined. The result was: (♀) +, 64; *v*, 67; *bb*, 20; *v bb*, 20; total 173; (♂) 164. This indicates clearly that *bb* is not located in the X-chromosome; for, if it were so, none of *bb* or *v bb* females would have appeared in the F_2 offspring. This fact was confirmed by a similar experiment in which a female with the *apricot* eye-color was used. The actual data were: (♀) +, 122; w^a , 112; *bb*, 42; $w^a bb$, 38; total 314; (♂) 272.

Next, in order to find out the chromosome to which the *bobbed* gene belongs, a *bobbed* female was mated to a *cardinal* (II) *plexus* (III) male, and the F_2 offspring were examined. The result was: (♀) +, 116; *cd*, 30; *px*, 30; *bb*, 40; *cd px*, 8; *cd bb*, 18; *px bb*, 20; *cd px bb*, 6; total 268; (♂) 248. This shows that *bobbed* segregates freely from either

cardinal or *plexus*, and accordingly, it must belong to the fourth chromosome.

A more direct evidence for the above-mentioned assumption was obtained from the phenomenon of pseudodominance. When a *bobbed* female is mated to a male deficient in a small V-shaped chromosome, i.e. haplo-IV male, the F₁ haplo-IV females are characterized by an extreme manifestation of the *bobbed* character. Further details concerning the *bobbed* mutant are presented in Part II, Chapter III.

IV. LINKAGE DATA AND LINKAGE MAPS

For preparation of the linkage maps, I have used not only my own data, but also those published by MORIWAKI. Naturally, however, the maps were constructed largely on my own data. The available data are listed in Table 3 and in Appendix II. This paper, however, is not primarily intended for details of such linkage data. The author is ready to provide anyone interested with such data if communicated with personally.

PART II. SPECIAL PROBLEMS *)

I. MUTATION FREQUENCY IN *D. ananassae*

As a preliminary requisite for the knowledge of the mutation frequency in any strand of chromosome, the point of spindle-fiber attachment should be found. Unfortunately, this has not yet been discovered with certainty for *D. ananassae*. Yet from the following experimental results, we can locate it approximately for each chromosome.

(A) *The X-chromosome*: As pointed out by GRAUBARD (1934) and by myself (1935b), it has been ascertained for the longer autosomes of *D. melanogaster*, that the coincidence value between two adjacent loci is very high in regions near the spindle-fiber attachment. By applying this relation to the data available for the X-chromosome of *D. ananassae*, we get the result shown in Table 4.

Contrary to the expectation that the middle portion of the genetic map should show the high coincidence, there was found no case where

*) After the manuscript was sent to the editor, it was found that the spindle-fiber attachment of the X-chromosome is located between the *mini-*

TABLE 3

Linkage data used in determining the map-distance and the order of genes. The column "loci tested" indicates the loci used in determining the map-distance, and the following three columns give totals of actual data. The column "test for order" shows loci by which the order of genes has been determined. In cases where no three-point experiment had been performed, the order was determined by consulting various data concerning two-point experiments. Such cases are put in brackets in the column "test for order". On some occasions, the order for the autosomal gene could be found by testing crossover individuals. The order of *c-Ila* was obtained by such means. The linkage maps constructed by the above procedure are represented in Fig. 5, together with other available maps.

THE FIRST CHROMOSOME

Symbol	Name	Locus	Loci tested	Cross-overs	Total	%	Test for order
<i>dp</i>	dwarf	0.0	<i>dp - v</i>	296	674	43.9	(<i>dp - v - ct</i>)
<i>N</i>	Notch	15.5	—	—	—	—	—
<i>w</i>	white	16.7	<i>N - w</i>	25	2002	1.2	<i>N - w - m</i>
<i>M-Ia</i>	Minute-Ia	30.5	<i>w - M-Ia</i>	89	713	12.5	<i>w - M-Ia - br</i>
<i>g</i>	garnet	37.5	<i>N - g</i>	207	944	21.9	<i>N - g - f</i>
<i>f</i>	forked	41.5	<i>g - f</i>	48	1243	3.9	<i>g - f - v</i>
<i>v</i>	vermillion	44.0	<i>f - v</i>	21	858	2.4	<i>g - f - v</i>
<i>m</i>	miniature	45.5	<i>v - m</i>	6	446	1.4	<i>g - v - m</i>
<i>dy</i>	dusky	46.2±	<i>f - dy</i>	7	129	5.4	<i>w - f - dy</i>
<i>ph</i>	purplish	65.0	<i>f - ph</i>	129	554	23.3	<i>f - ph - br</i>
<i>br</i>	broad	72.0	<i>ph - br</i>	38	554	6.9	<i>f - ph - br</i>
<i>sc</i>	scute	96.0	<i>br - sc</i>	134	559	24.0	<i>w - br - sc</i>
<i>y</i>	yellow	96.2	<i>sc - y</i>	1	539	0.2	<i>ph - sc - y</i>
<i>ac</i>	achaete	102.0	<i>br - ac</i>	125	433	28.9	<i>w - br - ac</i>
<i>Ir</i>	Interrupted	109.6±	<i>Ir - ct</i>	8	100	8.0	<i>m - Ir - ct</i>
<i>sn</i>	singed	112.7	<i>y - sn</i>	69	418	16.5	<i>y - sn - ct</i>
<i>ct</i>	cut	117.6	<i>sn - ct</i>	53	1090	4.9	<i>y - sn - ct</i>
<i>ab</i>	abnormal	163.6	<i>ct - ab</i>	99	215	46.0	<i>br - ct - ab</i>

ature and the purplish gene. Therefore, the inference advanced in Part II, Chapter I, can not be applicable at least to the X-chromosome. The detail concerning this problem will be published in a separate paper.

THE SECOND CHROMOSOME

Symbol	Name	Locus	Loci tested	Cross-overs	Total	%	Test for order
<i>cd</i>	cardinal	0.0	—	—	—	—	—
<i>c-IIa</i>	curved-IIa	1.0?	<i>C-IIa - Pt</i>	26	114	22.8	<i>c-IIa - Pt</i>
<i>sd</i>	spread	20.3±	<i>sd - Pt</i>	2	61	3.3	<i>sd - Pt</i>
<i>Pt</i>	Plexate	23.6	<i>cd - Pt</i>	1118	4743	23.6	<i>cd - Pt - tb</i>
<i>sk</i>	ski	23.6±	<i>Pt - sk</i>	0	1171	0.0	<i>Pt - sk</i>
<i>ss</i>	spineless	24.5	<i>Pt - ss</i>	9	960	0.9	<i>cd - Pt - ss</i>
<i>Off</i>	Off	37.8	<i>Pt - Off</i>	38	268	14.2	(<i>cd - Pt - Off</i>)
<i>tb</i>	tiny bristle	39.5	<i>Pt - tb</i>	310	1951	15.9	<i>cd - Pt - tb</i>
<i>e</i>	ebony	49.1	<i>Pt - e</i>	223	874	25.5	<i>cd - Pt - e</i>
<i>ms</i>	missing	56.7±	<i>Pt - ms</i>	?	154	33.1	(<i>Pt - Off - ms</i>)
<i>pk</i>	prickly	60.6	<i>Pt - pk</i>	407	1101	37.0	<i>cd - Pt - pk</i>
<i>sl</i>	slender	66.0±	<i>Pt - sl</i>	219	519	42.2	<i>cd - Pt - sl</i>
<i>M-IIa</i>	Minute-IIa	74.0?	<i>Pt - M-IIa</i>	178	344	50.3	<i>cd - Pt - M-IIa</i>

THE THIRD CHROMOSOME

<i>sk-III</i>	ski-III	0.0	<i>sk-III - Pm</i>	81	236	34.3	(<i>sk-III - Pm - Bb</i>)
<i>M-IIIa</i>	Minute-IIIa	19.0±	<i>M-IIIa - ba</i>	136	858	15.9	(<i>M-IIIa - ba - Px</i>)
<i>bn</i>	broken	22.0±	<i>bn - Pm</i>	84	630	13.3	(<i>bn - M-IIIb - Pm</i>)
<i>M-IIIb</i>	Minute-IIIb	26.5	<i>bn - M-IIIb</i>	31	695	4.5	(<i>bn - M-IIIb - Pm</i>)
<i>c-IIIa</i>	curved-IIIa	27.5±	<i>M-IIIb - c-IIIa</i>	4	364	1.1	<i>M-IIIb - c-IIIa</i>
<i>gp</i>	gap	33.5±	<i>M-IIIb - gp</i>	16	194	8.3	(<i>M-IIIb - gp - Pm</i>)
<i>ba</i>	balloon	35.0±	<i>ba - Pm</i>	0	286	0.0	<i>ba - Pm</i>
<i>Pm</i>	Plum	35.0	<i>gp - Pm</i>	8	573	1.4	(<i>M-IIIb - gp - Pm</i>)
<i>l-IIIa</i>	lethal-IIIa	35.0±	<i>Pm - l-IIIa</i>	0	?	±0.0	<i>Pm - l-IIIa</i>
<i>et</i>	extra	36.5±	<i>Pm - et</i>	12	793	1.5	<i>M-IIIb - Pm - et</i>
<i>Bb</i>	Barb	39.5	<i>Pm - Bb</i>	4	88	4.5	<i>Pm - Bb - px</i>
<i>px</i>	plexus	83.5	<i>Bb - px</i>	640	1454	44.0	<i>Pm - Bb - px</i>

THE FOURTH CHROMOSOME

<i>bb-IV</i>	bobbed-IV	0.0	—	—	—	—
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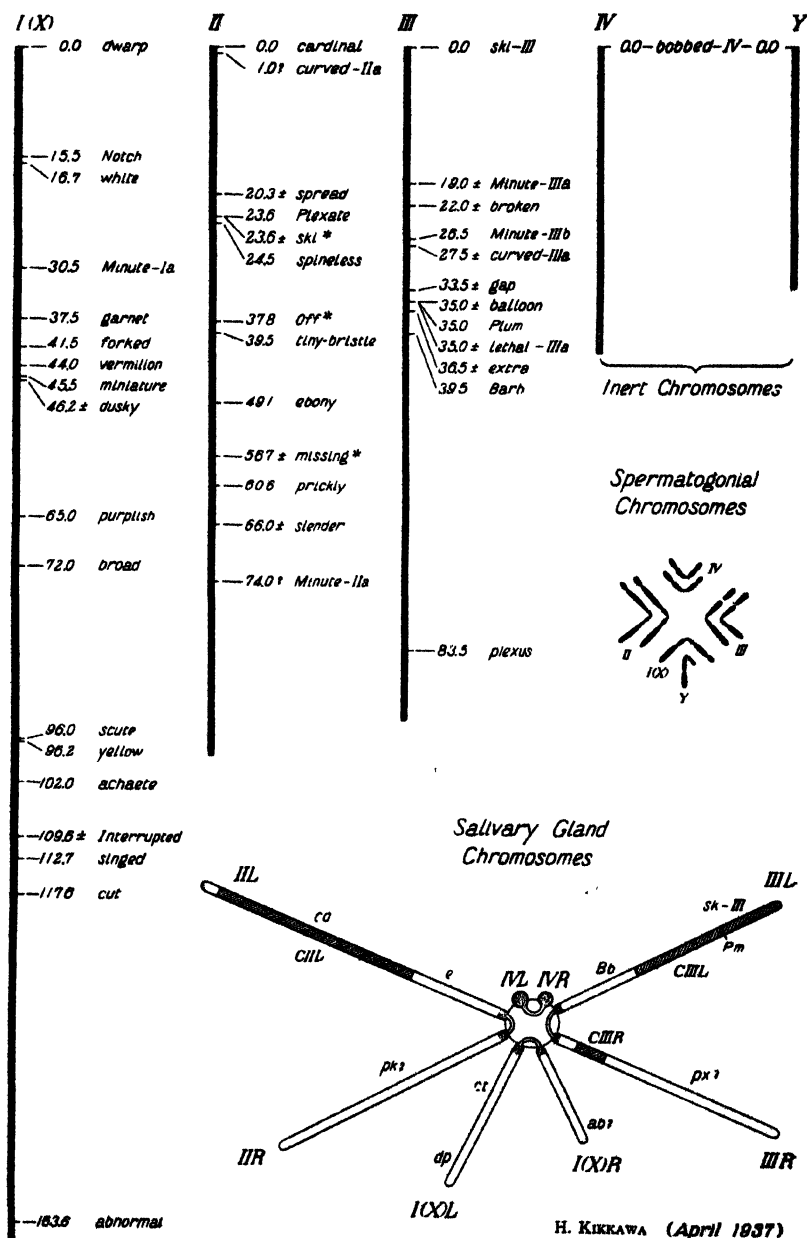
TABLE 4

Coincidence values in the X-chromosome of *D. ananassae*.

Cross	Recombination value (First region)	Recombination value (Second region)	Total value	Coincidence	Total number of flies
<i>w - g - m</i>	18.6	11.2	29.8	0.18	269
<i>w - f - m</i>	27.6	4.7	32.3	0.52	1204
<i>w - f - br</i>	24.5	23.3	47.8	0.91	3009
<i>w - br - sc</i>	30.3	24.0	54.3	0.72	559
<i>w - br - ac</i>	32.8	28.9	61.7	0.56	433
<i>g - f - v</i>	2.4	2.4	4.8	0.00	857
<i>g - v - m</i>	3.6	1.4	5.0	0.00	446
<i>f - ph - br</i>	23.3	6.9	30.2	0.11	554
<i>ph - sc - ct</i>	29.6	25.6	55.2	0.76	1615
<i>v - sn - ct</i>	16.5	4.8	21.3	0.61	418

the coincidence rises above or even reaches 1. This implies that, in the X-chromosome of *D. ananassae*, the spindle-fiber attachment is probably located to the right of the *cut* locus, hence all of the visible extant genes, except the *abnormal*, probably belong to one arm of X. A similar conclusion has been derived from an experiment concerned with high temperature (Table 5). There is no region in which the crossover frequency is changed markedly by high temperature.

(B) *The second chromosome*: I have been able to determine approximately the point of spindle-fiber attachment for this chromosome by using an inversion which is located in the left arm. The inversion CIIL, in the heterozygous state, suppresses crossing over entirely, in the regions from *cardinal* to *ebony*; but its effect does not extend to the *prickly* locus (Table 6).

FIG. 5. Chromosome maps of *D. ananassae*.

* The locus determined by MORIWAKI's data.

TABLE 5

The effect of high temperature on the crossover frequency in the X-chromosome of *D. ananassae*.

	Region tested		Total number of flies
	$w - f^2$	$f^4 - m$	
30° C	26.68 ± 0.59	4.24 ± 0.85	566
25° C	28.37 ± 0.56	5.02 ± 0.86	638
Diff./ σ	$1.59/0.85 = 1.87$	$0.78/1.21 = 0.64$	
	$ph - sc$	$sc - ct$	
30° C	28.52 ± 0.59	27.67 ± 0.58	589
25° C	30.11 ± 0.58	25.04 ± 0.55	631
Diff./ σ	$1.59/0.82 = 1.94$	$2.63/0.80 = 3.29$	

TABLE 6

The effect of an inversion (CIIL) on the crossover frequency in the second chromosome of *D. ananassae*

Cross	Non-crossover	Cross-overs	Total	Recombination percentage
<i>cd ss</i> /CIIL ♀ × <i>cd ss</i> ♂	1589+1698	0+1?	3288	±0.03
<i>cd tb</i> /CIIL ♀ × <i>cd tb</i> ♂	152+156	0+0	308	0.00
<i>cd e</i> /CIIL ♀ × <i>cd e</i> ♂	141+142	0+0	283	0.00
<i>cd ph</i> /CIIL ♀ × <i>cd ph</i> ♂	407+423	4+3	837	0.84

The individuals shown in the left side, in either the non-crossovers or the crossovers, involve the *cardinal* gene.

This result suggests that the spindle-fiber attachment is probably located in the region between *ebony* and *prickly*. The data of the coincidence for the second chromosome also reveal a similar fact (Table 7).

TABLE 7

Coincidence values in the second chromosome of *D. ananassae*.

Cross	Recombination value (First region)	Recombination value (Second region)	Total	Coinci- dence	Total number of flies
<i>cd - Pt - tb</i>	24.4	12.6	37.0	0.72	1035
<i>cd - Pt - c</i>	20.9	25.6	46.5	0.63	446
<i>cd - Pt - pk</i>	26.2	37.1	63.3	0.87	804

(C) *The third chromosome*: The *Plum* gene described in Part I, Chapter III, was used for this purpose. Judging from what has been revealed by microscopic examinations about the locus of *Plum*, it is very probable that all of the extant genes except *plexus* belong to the left arm of this chromosome.

(D) *The fourth chromosome*: No significant datum has yet been obtained concerning this chromosome.

Thus, among the mutations shown in Table 1, the more useful ones were selected and divided into seven chromosome groups. The number of such mutations is shown in Table 8.

It is noteworthy that, in *D. ananassae*, there is an undeniable tendency for the mutations to be restricted to one arm of each chromosome. This tendency may be recognized more clearly in the case where the frequency of recurrence of the same gene is taken into consideration (for example, the *white* gene of *D. ananassae* has so far been found four times). How can this tendency be accounted for?

It might be postulated that one arm of each chromosome of *D. ananassae* is formed by inert substance (KIKKAWA, 1935a). But, as shown in Part I, Chapter II and in the next chapter, all the arms of salivary gland chromosomes of this species except those of the fourth chromosome, have a great quantity of the "active" substance, so that the above assumption seems inadequate.

Those salivary chromosomes, however, differ from one another with respect to the length and the number of chromatin bands. The possibility is therefore not excluded that the length or the number of chromatin bands of a given strand may be proportional to the muta-

tion frequency in the respective strand. In order to see this point more minutely, comparison was made between the length of the active part of each chromosome strand and the frequency of mutation in the same strand. The results are shown in Table 8. In this table, the sex-linked mutations were dealt with separately from the autosomal gene because of the peculiar differences in the mode of appearance.

TABLE 8

Relation of the mutation frequency and the length of active part of the salivary chromosome or the number of chromatin bands, for a given strand, in *D. ananassae*, *D. melanogaster* and *D. virilis*. The available data for these species except *D. ananassae* have been taken from: *D. melanogaster* (Number of mutants, DIS. 3; Length of salivary chromosome, BRIDGES 1935); *D. virilis* (Number of mutants, CHINO 1936-1937; Length and number of chromatin bands of the salivary chromosome, FUJII 1936).

D. ananassae

Chromosome	XL	XR	IIL	IIR	IIIL	IIIR	(IVL + IVR)	Total
Number of chromatin bands (A)	154	91	310	242	234	253	7±	1291±
Length of active part (B)	100μ.	68	219	160	157	166	6	876
Number of mutants actually observed	23	1	10±	3±	10	1	2	50
Number of mutants calculated from (A)	15	9	8	6	6	6	0+	P=0.001583
Number of mutants calculated from (B)	14	10	8	6	6	6	0+	P=0.000408

D. melanogaster

Chromosome	X	IIL	IIR	IIIL	IIIR	IV	Total
Length of active part (B)	220 μ	215	245	210	275	15	1180
Number of mutants actually observed	174	59	87	39	62	17	438
Number of mutants calculated from (B)	..	59	67	58	76	4	$P=0.000000+$

D. virilis

Chromosome	X	II	III	IV	V	VI	Total
Number of chromatin bands (A)	286	403	282	292	291	14	1568
Length of active part (B)	171 μ	209	164	171	173	9	897
Number of mutants actually observed	68	26	20	27	30	7	178
Number of mutants calculated from (A)	..	35	24	25	25	1	$P=0.000000+$
Number of mutants calculated from (B)	..	32	25	26	26	1	$P=0.000000+$

As seen in this table, there is still some degree of discrepancy between the mutation frequencies and the length or the number of chromatin bands, of the active parts of salivary chromosomes. A similar fact is found also in *D. melanogaster* and in *D. virilis* (Table 8).

Thus, the nature of this phenomenon remains problematical; but the following possibilities may be considered:

(1) *Differential mutability*. As pointed out by MORGAN, BRIDGES and STURTEVANT (1925) and by MULLER (1928), the mutation frequency is not identical for all genes. If more stable genes accumulated in one arm of a chromosome than in the other, it would account for the above phenomenon very simply. As a matter of fact, such differential mutability has been found by BERG (1937*ab*) and SAPIRO (1936) in the first and second chromosomes of *D. melanogaster*.

(2) *Inert or sub-inert chromatin bands*. It seems unlikely that all parts of the salivary chromosome have the same physiological potency. As shown in Part II, Chapter VI, there are in *D. ananassae* one or a few chromatin bands which do not have any visible effect on the organism. Such chromatin bands, together with the achromatic parts which are located between them, may be regarded as representing inert or sub-inert substance, even though they are clearly visible in the nuclei of salivary gland cells. Thus the problem given above might be accounted for by the assumption that there are quantitative differences in the distribution of inert or sub-inert portions.

(3) *Undetectable genes*. It may be assumed that certain bands of salivary chromosomes represent peculiar genes which are hard to detect with ordinary methods — for example, the genes which control the intra-cellular metabolism. If such genes are accumulated in one arm of a chromosome, the frequency with which visible mutations occur in that arm should be less than in the other arm, even if the arms are equal in the absolute length or in the number of chromatin bands (genes).

At any rate, it seems noteworthy that in *D. ananassae* the mutation frequency of visible genes is not proportional to the length or the number of chromatin bands of the active part of the salivary chromosome.

II. INERT CHROMOSOMES

In the beginning of this study, I had expected from the ovarian chromosome type of this species, to find that it has four large linkage groups. Soon afterwards, however, this proved not to be the case. All of the mutations thus far analysed except the *bobbed-IV* gene, are included in three linkage groups only. The actual number of muta-

tions found are: $X = 27$ including multiple alleles, $II = 23$, $III = 15$, $IV = 2$.

From this fact, I assumed that one of the autosomes was virtually inert. As shown in my previous papers (KIKKAWA, 1936a, 1937a), this assumption has been verified in various ways.

The evidence for this, first of all, has been derived from the examination of the salivary gland chromosomes. As stated before, only six chromosome strands instead of eight radiate from the chromocenter. Subsequent studies have proved that these six strands represent the paired arms of the first, second and third chromosomes. Therefore, the remaining autosome pair which are present in the chromocenter as a small bipartite mass, should be regarded as the two arms of the fourth chromosome. This has been confirmed more definitely by the study of the relation between the small pair and the nucleolus, and by the examination of a III-IV mutual translocation (*Plum*). The fact that the fourth chromosome in a salivary gland cell is very small in relative size as compared with that in a germ cell, is readily accounted for by the assumption that this chromosome contains a great quantity of inert substance, i.e. heterochromatin of HEITZ (HEITZ, 1933ab, PAINTER, 1934).

A more direct confirmation of the above statement was made by the discovery of haplo-IV individuals. In the autumn of 1935, I carried out some experiments utilizing X-rays and supersonic vibrations, in order to get individuals resulting from primary non-disjunction. The results are shown in Table 9, together with the control datum.

It was of interest that many *Minute* flies appeared in these experiments. Some of them were mosaics with respect to the *Minute* character (Fig. 6a). Of eight *Minute* females examined cytologically, five were deficient in one of the small V-shaped chromosomes (Fig. 2d, e; Fig. 6a; Pl. V a).

Many such haplo-IV individuals have since been obtained from various stocks, especially the XXY stock. They show features common to similar flies found in other species such as *D. melanogaster* and *D. virilis*; namely the body-size is smaller, the bristles are relatively shorter and more slender; the body-color is paler, the wings are slightly extended and with blunt tips. Moreover, they are characterized by frequent sterility (especially in females), slow development

TABLE 9

Experimental results concerning primary non-disjunctions. X-rays: Cross, $w \text{ ♀} \times + \text{♂}$ (virgin females emerged within one day); Condition, 35 KV. 2.2 ma. 16 cm. Non-filter. COOLIDGE tube with tungsten target. Supersonic vibrations: Cross, $w \text{ ♀} \times + \text{♂}$ (full grown larvae); Condition, Crystal oscillator. About 25000 vibrations per second. * Mosaics. † All exceptional males were sterile.

	Regular		Exceptional			♀ ♂ %			Total
	♀	♂	♀	♂	%	♀	♂	%	
Control	1836	1553	1	1	0.06	8	1	0.26	3400
X-rayed (60 min.)	2362	2048	0	2	0.12	6+1*	3	0.23	4422
X-rayed (90 min.)	1566	1499	0	7	0.23	9	6	0.49	3087
Supersonic vibration	2052	1562	0	0	0.00	3+1*	1*	0.14	3619
Total. . . .	7816	6662	1	10	0.08	26+2*	10+1*	0.27	14528

and heavy mortality. The genetic behavior of the haplo-IV males of *D. ananassae* is described in the next chapter.

The fact that the fourth chromosome does not exert a great influence on the phenotype and viability, has been further proved by the discovery of triplo-IV individuals. As will be shown later, such flies are obtained frequently in the progeny of an XXY female; but, except by microscopic examination, they are hardly distinguishable from the normal type.

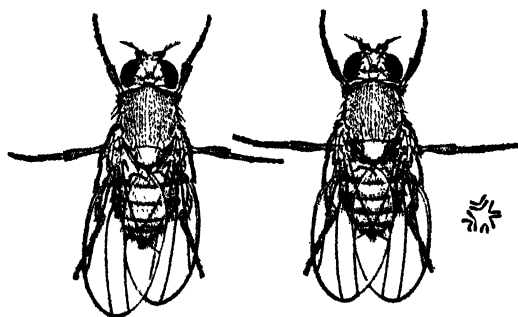


FIG. 6. A mosaic female with respect to haplo-IV and normal type (left figure), and a haplo-IV female (right figure).

Before concluding this chapter, a few statements may be

added as to the part which the inert chromosomes exert possibly on the organism. Geneticists and cytologists are prone to consider such important problems as the phylogeny of related species and the number and size of linkage groups, merely on the basis of the chromosome types found in the germ or ordinary somatic cells. Strictly speaking, however, such problems can not be solved thoroughly without exact information as to the inert parts of the chromosomes of the species in question. As a matter of fact, the presence of such inert chromosomes or inert regions of chromosomes has been detected not only in *Drosophila*, but also in other organisms as *Sciara* (METZ, MOSES, HOPPE, 1926) and *Sphaerocarpus* (KNAPP, 1935). Whether the inert chromosome or the inert region of chromosome indicates the process of degeneration or creation of the genic materials, remains to be solved by future studies, though most investigators seem to lean toward the former opinion.

III. SECONDARY SEX-CHROMOSOMES

This chapter is to be devoted to the fact that there are certain homologous regions between the sex-chromosomes and the autosomes.

(A) This idea was first suggested by experiments concerning haplo-IV individuals (Table 10).

TABLE 10

Experimental results concerning haplo-IV individuals of
D. ananassae.

Cross	Normal		Haplo-IV		Total
	♀	♂	♀	♂	
Haplo-IV ♀ × + ♂	87	63	27	25	202
+ ♀ × Haplo-IV ♂	992	440	264	581	2277

Contrary to the normal result in the cross of haplo-IV ♀ × + ♂, in the cross of + ♀ × haplo-IV ♂, the number of haplo-IV males was about twice that of the corresponding females, while the number of normal type males was about a half that of the corresponding fema-

les. Various hypotheses might be postulated for this unexpected ratio; but the result can be completely explained by the conception that there is a homologous part between the Y-chromosome and the fourth chromosome. Supposing that $x\%$ of the solitary fourth chromosome undergo synapsis with the Y-chromosome in the meiotic divisions of haplo-IV male (Type 1 in Fig. 7), the offspring may segregate in the following ratios (here $y = 1 - x$):

Normal females	$(2x + y)/4$
Normal males	$y/4$
Haplo-IV females	$y/4$
Haplo-IV males	$(2x + y)/4$

Calculating the values x and y from the actual data, we get: $x = 38.2\%$, $y = 61.8\%$. The deviation between the theoretical and experimental results may be due to low viability of haplo-IV individuals. The schema illustrating the above relation is shown in Fig. 7.

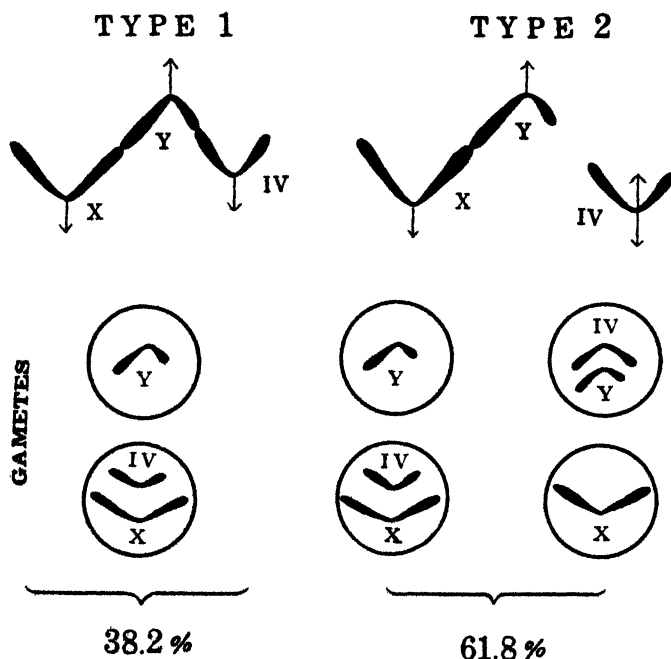


FIG. 7. Schematic figure illustrating the relation between the sex-chromosomes and the fourth chromosome in a haplo-IV male.

(B) The second evidence is based on the behaviors of the fourth

chromosome in an XXY female. In Table 11, are represented the summarized results of the secondary non-disjunction of this species. Some of the XXY females used in the experiment were derived from different sources, but they showed quite similar results.

TABLE 11

Experimental results concerning secondary non-disjunction of
D. ananassae.

Regular		Exceptional		Haplo-IV		Total
♀	♂	♀	♂	♀	♂	
584	520	1	2	147	123	1377

As seen in this table, notwithstanding the fact that the frequency of the secondary non-disjunction is very small in this species (about 0.2%), there are found many *Diminished* or haplo-IV flies among the

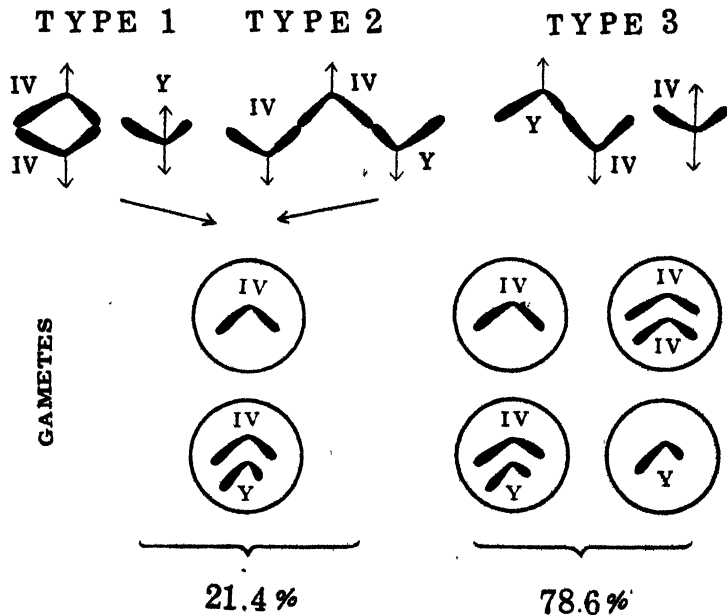


FIG. 8. Schematic figure illustrating the relation between the Y-chromosome and the fourth chromosomes in an XXY female.

offspring. This unexpected result may be also accounted for by the conception that an extra Y-chromosome in the XXY female frequently synapses with one of the fourth chromosomes in the meiotic divisions. The schema illustrating this relation is shown in Fig. 8.

Considering the expected genotypes and their frequencies in the offspring of an XXY female from this figure and the available data, we get: (Here the non-disjunctional flies were omitted).

(x = Type 1 + Type 2; y = Type 3)

X X IV IV + X Y IV IV; $(2x + y)/4 = 30.4\%$

X X Y IV IV + X Y Y IV IV; $(2x + y)/4 = 30.4\%$

X X IV IV IV + X Y IV IV IV; $y/4 = 19.6\%$

X X Y IV + X Y Y IV; $y/4 = 19.6\%$

It is easy to discriminate the haplo-IV individuals from the normal ones, but the XXY and triplo-IV females can not be distinguished phenotypically from the normal sibs. Microscopic examination was made of the females of the normal phenotype. But, owing to the difficulty of discriminating the XXY and triplo-IV complexes, doubtful cases were omitted. Thus, of all the 15 females examined, 8 were of the normal type (Fig. 2 *a, b*), 4 were of the XXY type (Fig. 2 *h, i*; Pl. Vc) and the remaining 3 were of the triplo-IV type (Fig. 2 *j, k*; Pl. Vd). On the other hand, all of the ten haplo-IV females examined cytologically had an extra Y-chromosome (Fig. 2 *f, g*; Pl. Vb). This fact accords with the theoretical expectation.

(C) The third evidence is based entirely on cytological observations. In the oögonial metaphase of a haplo-IV female involving an extra Y, the solitary fourth chromosome tends to lie in contact with the Y-chromosome. But, this tendency is observed in one arm of the chromosome only; the other arms show an apparent tendency to repulse each other (Fig. 2 *f, g*; Pl. Vb).

From these facts it has been assumed that the distal part of the longer arm of the fourth chromosome is homologous to the shorter arm of the Y-chromosome, though KAUFMANN's figures (1937*b*) imply that it is homologous to the distal part of the longer arms of the Y-chromosome. Such a partial affinity between the Y-chromosome and the fourth chromosomes, is found also in the XXY female (Fig. 2 *h, i*; Pl. Vc), haplo-IV male (Fig. 2 *c, p*), and even in the normal male (Fig. 2 *c, m*).

In connection with this, it is very interesting to review KAUF-

MANN's (1936*ab*, 1937*ab*) cytological works on *D. ananassae*. According to these, the fourth chromosomes are attached to the nucleolus or nucleoli with the distal ends of their longer arms during the early prophase stages in ganglion cells of both sexes. In the male, however, the Y-chromosome forms the third member of the group which is associated with the nucleolus. These facts seem to afford further evidence for the conclusion on cytological grounds.

(D) A crucial corroboration for the above statement has been obtained by studies of the *bobbed* character. As shown in Part I, Chapter III, this mutant is located in the fourth chromosome, and does not appear in the male. Further, the data on the III-IV mutual translocation (*Plum*), support the view that *bobbed* lies in the distal portion of the longer arm.

In order to investigate the question whether or not the Y-chromosome is really responsible for the *bobbed* manifestation (MORIWAKI's papers contain no conclusive experiment of this point), I made the following experiment. Virgin *v bb* females irradiated with X-rays, were mated to *bobbed* males. Table 12 shows the result.

TABLE 12

Experimental results concerning primary non-disjunction of *D. ananassae* in the case where the *bobbed* gene was used (35 KV. 2.2 ma. 16 cm. 35 min. Non-filter. COOLIDGE tube with tungsten target).

Regular		Exceptional		Total
♀	♂	♀	♂	
1308	1282	1 (+)	8 (<i>bb</i>)	2599

In this experiment, one XXY (strictly speaking XXY IV^{bb} IV^{bb}) female and eight XO (XO IV^{bb} IV^{bb}) males, which were the result of the primary non-disjunction, were obtained. Although the exceptional female died without leaving any progeny, she apparently had normal bristles.

Especially noticeable were the XO males. They all had very slender and short bristles. Moreover four of them had scaly sclerites—a character often found in the *bobbed* females but never observed in the corresponding males.

Furthermore, I obtained recently an XXY female from the *cd px bb-IV(bb)* stock. Experimental results utilizing this exceptional female, accorded with the theoretical expectation in both the genetical and the cytological aspects (Table 13).

TABLE 13

The progeny of mating XXY IV^{bb} IV^{bb} ♀ × XY IV^{bb} IV^{bb} ♂. Since it is difficult to discriminate the haplo-IV^{bb} females from the *bb* sibs, the number of such females shown in the table may not be quite accurate.

	Regular			Haplo-IV		Total
	♀ +	♂ <i>bb</i>		♀	♂	
Genetical	56	89 ±	143	16 ±	27	331
Cytological	XXY IV IV=6	XX IV IV=6 XX IV IV IV=3	..	XXY IV=3	..	18

It has thus become clear that the Y-chromosome possesses a dominant allele of the *bobbed* character. However, for the maintenance of the above idea, it is necessary to assume that the region in which the *bobbed* mutant is located, is entirely missing in the X-chromosome of *D. ananassae*. As previously shown by HEITZ (1933*ab*) and by KAUFMANN (1934), in *D. melanogaster*, *D. funebris*, *D. virilis* and in *D. hydei*, the nucleolus develops in the X- and Y-chromosome, in which the *bobbed* character is located. Contrary to this general rule, the X-chromosome of *D. ananassae* lacks the nucleolus-forming region and only the Y- and the fourth chromosomes are concerned in the formation of the nucleolus (or nucleoli). These facts lead us to the conclusion that the nucleolus-forming region is closely connected with the *bobbed* locus. This has been ascertained for *D. melanogaster* by KAUFMANN (1934), MORGAN, BRIDGES and SCHULTZ (1935) and by NEUHAUS (1936).

While studying the genetical behaviors of the haplo-IV males which were derived from the XXY female, another noteworthy phenomenon was noted. As previously stated, it is assumed that each of those haplo-IV males possesses an extra Y-chromosome. When

they are mated to normal females, the offspring segregate in an abnormal ratio as shown in Table 14.

TABLE 14

The progeny of mating XX ♀ × XXY haplo-IV ♂.

Normal		Haplo-IV		Total
♀	♂	♀	♂	
49	210	140	26	425

The result shown in this table is quite different from that of the ordinary case of similar mating (Table 10). Namely, the number of the haplo-IV females is larger than that of the corresponding males, whereas the number of the normal females is much smaller than that of the corresponding males. This result implies that the X-chromosome has a tendency to segregate from the fourth chromosome in the meiotic divisions of an XXY haplo-IV male. Hence, the X-chromosome probably has a region which is homologous to a part of the fourth chromosome.

The relation stated above may be presented schematically as in

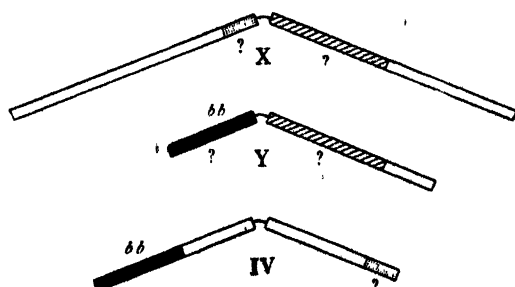


Fig. 9, though it is still speculative in either the locus or the distance of homologues.

The most noteworthy fact established in this chapter is that there are some homologous regions between the sex-

chromosomes and the autosomes. Hence, the autosomes behave as if they were the sex-chromosomes in certain cases. I suppose that a similar phenomenon occurs in the related species *D. bipunctata* and *D. montium* too (KIKKAWA, 1936b). Moreover, this fact may throw light on the problem of the origin of the sex-chromosomes as well as

of the duplication process of genic materials in one genom (see DOBZHANSKY and TAN, 1936).

IV. SPONTANEOUS CROSSING OVER IN THE MALE

It is well known that crossing over is very rare in the male of *Drosophila* in the normal condition, though it is considerably increased by high temperature and X-rays (FRIESEN, 1933; KIKKAWA, 1933; MORIWAKI, 1935c; PATTERSON and SUCHÉ, 1934; WHITTINGHILL, 1937, etc.). This holds true for *D. ananassae* (MORIWAKI, 1936a). But, a strain was found recently in which spontaneous crossing over occurs very frequently in the male (KIKKAWA, 1937b). The detailed analysis of this strain is now in progress, but it is already clear that this abnormal phenomenon is due to one or more dominant genes located probably in the third chromosome, which are not accompanied by any visible chromosome aberrations in either germ or salivary gland cells. In Table 15, the experimental results are given for this gene in the third chromosome, together with the control data.

TABLE 15

Experimental results concerning male crossing over in *D. ananassae*. Individuals showing in the left side, in either the non-crossovers or the crossovers, involve the *plexus* gene *En* = Enhancer or Enhancers of male crossing over.

Cross	Number of cultures	Non-cross-overs	Cross-overs	Total	Recombination percentage
<i>px</i> ♀ × <i>Bb/px, En</i> ♂	8	424 + 458	15 + 21	918	3.92
<i>px</i> ♀ × <i>Bb/px</i> ♂	8	474 + 513	0 + 0	987	0.00
<i>px</i> ♀ × <i>Bb px/+ , En</i> ♂	3	133 + 134	1 + 3	271	1.48
<i>px</i> ♀ × <i>Bb px/+</i> ♂	7	336 + 315	0 + 0	651	0.00
<i>px</i> ♀ × <i>Pm/px, En</i> ♂	16	648 + 663	89 + 84	1484	11.66
<i>px</i> ♀ × <i>Pm/px</i> ♂	31	1419 + 1432	0 + 0	2851	0.00
<i>px</i> ♀ × <i>Pm px/+ , En</i> ♂	15	518 + 592	51 + 44	1205	7.88
<i>px</i> ♀ × <i>Pm px/+</i> ♂	13	647 + 611	0 + 0	1258	0.00

The strain in question was originally found at the end of 1936 in the *plexus* stock. As shown in this table, the frequency of crossing over in the male involving the enhancer (or enhancers) in a heterozygous state, is extraordinarily high as compared with the same in the zynormal male. The crossover frequency, however, varies considerably with culture, even when brothers have been used. For example, in the mating of $px \text{ } \varnothing \times Pm/px$, $En \text{ } \delta$ or $px \text{ } \varnothing \times Pm \text{ } px/+$, $En \text{ } \delta$, the crossover value varies from nearly zero to 20 percent (Table 16). It is, therefore, quite possible that there are many cultures in which no crossing over actually occurred in spite of its possibility, and such cases were wholly omitted from the results in Tables 15 and 16.

TABLE 16

Variations in the crossover frequency in the mating of
 $px \text{ } \varnothing \times Pm/px \text{ } E, n \text{ } \delta$, and of $px \text{ } \varnothing \times Pm \text{ } px/+$, $En \text{ } \delta$.

Recombination percentage	Number of cultures	Recombination percentage	Number of cultures
0.1 — 2.0	5	10.1 — 12.0	4
2.1 — 4.0	3	12.1 — 14.0	2
4.1 — 6.0	3	14.1 — 16.0	2
6.1 — 8.0	3	16.1 — 18.0	4
8.1 — 10.0	3	18.1 — 22.0	2

In order to know the linkage group to which the enhancer (or enhancers) belongs, the following experiment was carried out. One $Pm \text{ } px$ male resulting from crossing over in the male, was mated to a wild type female, and the sons with Pm were mated separately to the px females. Similarly, one $Pm \text{ } px$ female derived from the male crossing over, was used as the mother in a reciprocal mating. In the former case, 10 out of 14 sons gave crossover individuals among their offspring, while in the latter, 6 out of 13 sons gave the crossover individuals. This fact suggests that the enhancer (or enhancers) is located in the third chromosome. The absence of male crossing over in the four cultures in the former case, may be due either to previous crossing over in the grandfather or to failure of crossing over in spite of its possibility.

The effect of the enhancer or enhancers on the crossover frequency in the second chromosome of the male, is negative as far as the available data show (Table 17).

TABLE 17

Effect of the enhancer or enhancers of male crossing over on the crossover frequency in the second chromosome. $cd\ e\ \varphi \times cd\ e/+$, $En\ \delta$.

Non-crossovers		Crossovers		Total
$cd\ e$	+	cd	e	
208	212	0	0	420

Furthermore, it seems that the crossover frequency in the male does not change markedly with the age of the father. This conclusion has been derived from an experiment in which the males involving the enhancer (or enhancers) were mated to virgin females several days after the first mating. Table 18 shows the result for the third chromosome.

TABLE 18

Variation in crossing over in relation to age of the male involving the enhancer or enhancers. $px\ \varphi \times Pm\ px/+$, $En\ \delta$.

Period	Non-crossovers		Crossovers		Total	Recombination percentage
	$Pm\ px$	+	Pm	px		
1—4	125	131	11	7	274	6.57
5—10	77	82	5	6	170	6.47

In connection with this, it is noteworthy that MORIWAKI (unpublished) has obtained a similar enhancer of crossing over which is located in the second chromosome of *D. ananassae*. According to personal correspondence from him, this gene called *Minute-IIb* by him, affects the crossover frequency in the second chromosome only.

At present, it is difficult to state definitely what kind of mechanism is responsible for the occurrence of male crossing over. From the fact that crossover individuals are found in a small portion of the cultures tested, several investigators (FRIESEN, 1936; NEUHAUS, 1936; STERN and DOAN, 1936; WHITTINGHILL, 1937) support the view that crossing over takes place in stages earlier than maturation. This may be true; but it seems equally possible that such a result as stated above is due to crossover enhancers which were introduced by chance in some cultures. NEUHAUS (1936) had denied the presence of such enhancers on genetical grounds, while FRIESEN's (1934) experimental result reveals clearly that there is, at least, one strain in which the male crossing over is induced by X-rays or by high temperature. Recent experiments of WHITTINGHILL (1937) suggest also the presence of similar genes.

V. RACIAL INVERSIONS

In 1936 (*b*), KAUFMANN reported an interesting fact concerning the salivary gland chromosomes of *D. ananassae*. There have been detected four inversions in hybrids between individuals of the Tuscaloosa and one of the Japanese strains. Two of the inversions "terminal" and "basal" occur in all the hybrids, while the remaining two "sub-terminal" and "median" exist in a heterozygous state in one of the parental strains. Of these four inversions, the terminal inversion has been studied minutely by him because of its peculiarity.

Previous to receiving his paper I had observed a similar inversion in one of the Japanese strains (Okiwana-T strain). A comparison of his figures with mine has shown that KAUFMANN's report of inversion are identical with mine.

Thus the question arises as to whether or not KAUFMANN's inversion was derived from the hybridization between the American and Japanese strain as he states. In order to solve this problem, minute examination of the salivary chromosomes in every strain preserved in this laboratory has been made. For this purpose, full-grown female larvae were taken from a mass culture of a given strain, and the salivary chromosomes were examined by the usual method. As stated by GORDON (1936) a mass culture may be regarded as representing a wild population on a small scale. The strains used are shown in Table 19.

TABLE 19

Different strains of *D. ananassae* used in detecting racial inversions.

Strain somewhat symbolized	Locality	Collector	Date of Collection	Kinds of chromosome aberrations detected
Tuscaloosa-K	Alabama	KAUFMANN, B. P.	33?	<i>CIIL, CIIR, CIIL, EIIL</i>
Tôkyo-M	Tôkyo	MORIWAKI, D.	31g	<i>CIIL, CIIL, CIIR</i>
Naka-Iwôtô-D	Iwôtô Isles	DAIDÔ, K.	36l	<i>CIIR</i>
Amami-Ôsima-O	Ryukyu Isles	ÔBA, H.	36j	<i>CIIL, CIIL</i>
Okinawa-T	Ryukyu Isles	TÔMA, T.	36j	<i>CIIL, CIIL, CIIR</i>
Isigakizima-M	Ryukyu Isles	MASAKI, J.	34j	<i>CIIL</i>
Taihoku-A	Formosa	AOTA, S.	34k	<i>CIIL</i>
Taihoku-K	Formosa	KOMAI, T.	32j	..
Taihoku-O	Formosa	ÔMORI, N.	34k	<i>CIIL-O, CIIR</i>
Taihoku-S	Formosa	SHIRAKI, T.	34j	<i>CIIR</i>
Taihoku-T	Formosa	TAKAHASHI, R.	34l	..
Sintiku-Y	Formosa	YAMANAKA, M.	36k	<i>CIIL</i>
Tainan-O	Formosa	Ô., U.	36j	<i>CIIL</i>
Takao-O	Formosa	ÔTA, K.	36k	<i>CIIL, CIIL</i>
Shanghai-H	Shanghai	PENG, F. T	35i	<i>CIIL, CIIL, EIIL</i>
Shanghai-T	Shanghai	TOMITA, G.	36h	<i>CIIL, CIIR, EIIR, EIIL</i>

Thus it has been found that there are, at least, five different inversions in wild populations of *D. ananassae*. Three of these were detected in more than two different strains, and each of the remaining two was found in a single strain. The schematic figures of these inversions are shown in Fig. 10.

(A) C I I L

This inversion corresponds with KAUFMANN's subterminal inversion.

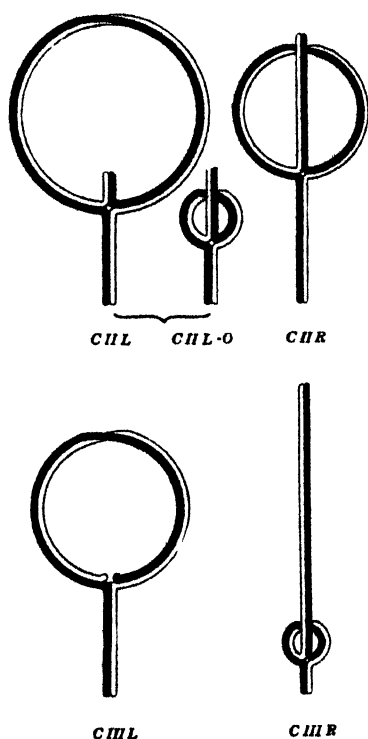


FIG 10 Schematic figures of inversions detected in wild populations of *D. ananassae*

It suppresses the crossing over, in a heterozygous state, in the region from *cardinal* to *ebony*, but its effect does not extend to the *prickly* gene (Table 6). On both genetical and cytological grounds it is clear that the inversion is viable in a homozygous state. Plate I g, II a shows examples of this inversion: C I I L is distributed widely in wild populations of such distant places as America, Japan and China. The strains involving it are as follows: Tuscaloosa-K (15/23), Tokyo-M (2/10), Amami-Osima-O, Okinawa-T, Takao-O, Shanghai-H, Shanghai-T (2/8) (here the number of individuals involving C I I L in a heterozygous state is shown, when recorded, before an oblique line, and the total number tested is given after that line in brackets).

The strains in which C I I L has not yet been detected, are Naka-Iwôtô-D (0/18), Taihoku-K (0/10), Taihoku-O (0/6), Taihoku-S (0/10), Taihoku-T (0/10), Sintiku-Y (0/7). The absence of C I I L in those strains can not be considered certainly until more extensive tests have been performed.

(B) C I I L — O

This inversion is detected in only one strain, the Taihoku-O (2/6). A small proximal portion of the left arm of the second chromosome is inverted as shown in Pl. I h, 1. The distal breakage point of this inversion is located very near the proximal breakage point of C I I L. The test for viability in a homozygous state has not yet been performed.

(C) K. median inversion is correspondent to this inversion. It has been reported so far only in the Tuscaloosa-K strain (15/23) in com- IL. Figures of this inversion are shown in Pl. I j and Pl. I k. Whether or not it is viable in a homozygous state has to be shown.

(D) T. dependent to the terminal inversion by KAUFMAN. Its peculiarity, special attention has been paid to the distribution of this inversion in wild populations. In the salivary chromosome of several larvae taken from a strain involving CIIL, are observed carefully, there are always found two types of chromosome with respect to the configuration of the left arm of the third chromosome. These I shall call A and B.

The same scheme representing the A type is shown in Pl. I a. The case where the distal portion of the chromosome in the third chromosome is inverted. The individual heterozygous for A and B, there is a racket-shaped configuration as given in Pl. I c, d; Pl. I e. The result analysed for each strain is represented in Table 20.

TABLE 20

Distribution of CIIL in wild populations.

	Strains involving CIIL			
	AA	AB	BB	Total
Tuscaloosa	8	10	5	23
Tôkyô	3	6	1	10
Amanohashi	4	2	1	7
Okinawa	3	2	1	6
Isigahara	6	3	1	10
Taipei	1	1	1	3
Taipei	2	4	0	6
Tainan	1	1	2	4
Takao	1	3	1	5
Shanghai	4	4	8	16
Total	33	36	21	90
Gen.				32

Strains not involving <i>CIIL</i>				Total
Strain	AA	AB		
Naka-Iwôtô-D	17	0		17
Taihoku-K	10	0		10
Taihoku-S	10	0		10
Taihoku-T	10	0		10
Sintiku-Y	7	0		7
Shanghai-T	27	0		27
Total	81	0		81

Of the 16 strains tested, 10 contain *CIIL*. It has been found that the chromosomes in the strain in which no *CIIL* was present were always to the A type. The fact that A is common to all the strains in which *CIIL* was detected has been ascertained in all the strains in which *CIIL* was detected, although in a few cases, e.g. Shanghai-H.

The genetical behavior of this inversion has not yet been thoroughly studied. But, from several experiments concerning the inheritance (see Part I, Chapter III), it is already clear that *CIIL* is even in an individual heterozygous for A and B.

(E) *C III R*

This inversion corresponds to KAUFMANN's *bac* inversion, which is found very near the proximal end of the right arm of the X chromosome, and is viable in a homozygous state. The results of the tests are shown in Pl. I *b, e, f* and Pl. II *e, f*.

This inversion, as well as *CIIL* and *CIIL*, is distributed in wild populations of distant localities as shown by the following results:

CIIL present: Tôkyo-M (6/10), Naka-Iwôtô-D (10/10), Taihoku-T (10/10), Taihoku-O (4/6), Taihoku-S (2/10), Shanghai-T (10/10).

CIIL absent: Tuscaloosa-K (0/23), Taihoku-T (0/10), Sintiku-Y (0/7).

The results of investigation on the relationship between the three mentioned inversions are shown in Table 21.

The results show that: (1) The inversions involving *CIIL* and *CIIL* are constant in kind: for example, Tôkyo-M and Taihoku-K or Taihoku-S have the same kinds of inversions, *CIIL*, *CIIL* and *CIIL*, while

TABLE 21

Relationship between the racial inversions. CIIL-O and CIIR were omitted from the table, since each was detected in only a single strain.

Strains	Kinds of combinations									Total	Kinds of inversions
	+	<i>CIIL</i>	<i>CIIL</i>	<i>CIIR</i>	<i>CIIL</i> <i>CIIL</i>	<i>CIIL</i> <i>CIIR</i>	<i>CIIL</i> <i>CIIR</i>	<i>CIIL</i> <i>CIIR</i>	<i>CIIL</i> <i>CIIR</i>		
Tuscaloosa-K	6	7	2	0	8	0	0	0	23	<i>CIIL</i> , <i>CIIL</i>	
Tôkyo-M	3	1	0	0	0	0	5	1	10	<i>CIIL</i> , <i>CIIL</i> , <i>CIIR</i>	
Naka-Iwôtô-D	12	0	0	5	0	0	0	0	17	<i>CIIR</i>	
Taihoku-K	10	0	0	0	0	0	0	0	10	..	
Taihoku-O	2	0	0	0	0	0	4	0	6	<i>CIIL</i> , <i>CIIR</i>	
Taihoku-S	8	0	0	2	0	0	0	0	10	<i>CIIR</i>	
Taihoku-T	10	0	0	0	0	0	0	0	10	..	
Shanghai-T	4	1	0	2	0	1	0	0	8	<i>CIIL</i> , <i>CIIR</i>	
Total	55	9	2	9	8	1	9	1	94	..	

Taihoku-T strain has none of them; (2) CIIL is found quite irrespective of the presence or absence of CIIL and CIIR (e.g. Tuscaloosa-K, Shanghai-T), while CIIL and CIIR are found together in most cases (e.g. Tôkyo-M, Taihoku-O).

As to the question whether these inversions are of monophyletic or polyphyletic origin, the latter seems more plausible than the former. As the ground for this assumption, PATTERSON, et al. (1934), and especially BRIDGES and LI (1937), have pointed out that translocations or inversions are liable to occur at a definite point of the chromosome. Further, it is well known that inversions occur sporadically in wild populations of *Drosophila* species (FROLOVA, 1936; STURTEVANT 1919, 1931). And also GERSHENSON (1934) and GRÜNEBERG (1936, 1937) have found cases of the occurrence of reinversions, though they are very rare.

In *D. ananassae* too, it is likely that there are one or a few weak points in each arm of the longer autosomes where the breakage of the chromosome often occurs, and this gives rise to the same type of inversion independently in different strains.

The male crossing over noted in the preceding chapter seems to have something in common with the above phenomenon. Critical study of this point is now being made.

At any rate, the presence of inversions characteristic of each race as stated above, is of interest and importance in connection with the problem of differentiation of species. Similar definite types of inversions have been found in the hybrids between *D. melanogaster* and *D. simulans* or between *pseudoobscura* groups (PÄTAU, 1935; KOLLER, 1936; TAN, 1935). The chromosome abnormality observed in such hybrids as that between *D. pseudoobscura* and *D. miranda* (DOBZHANSKY and TAN, 1936) or between *D. azteca* and *D. athabasca* (STURTEVANT and DOBZHANSKY, 1936) may be regarded as indicating a higher step of specific differentiation.

VI. EXTRA CHROMATIN BANDS IN SALIVARY GLAND CHROMOSOMES

While studying the inversions shown in the preceding chapter with the salivary method, I have noticed that there are some strains in which the landmarks of the distal end of certain strains are different from those of the normal type. Such a structural difference has so far been ascertained in three different strains, i.e., the left arm and the right arm of the second chromosome, and the left arm of the third chromosome. These chromosome aberrations may be called EIIL (Extra, Second, Left), EIIR and EIIL respectively.

(A) EIIL

In the normal condition, the distal end of this chromosome strand extends into a fan-shape, and is marked by about eight obscure bands (Fig. 3; Fig. 11 *a*; Pl. II *g*). In the EIIL strain, an additional conspicuous band is attached to the end of this as shown in Fig. 11 *b, c, d* and Pl. II *a, h*. This chromosome aberration has been detected in two different strains, the Tuscaloosa-K (1 : 5 : 12) and the Shanghai-H (2 : 6 : 16) strains. (Here the numbers given in brackets indicate homozygous EIIL, heterozygous EIIL and normal type respectively)

(B) EIIR

This chromosome aberration was detected quite recently in the Shanghai-T strain (1 : 4 : 10). A conspicuous nipple-like band and a minute band are attached to the distal end of the normal type chromosome (Fig. 11 *e, f, g, h*).

(C) EIIIL

This has been found also in the Shanghai-T stock (3 : 12 : 12). A



FIG. 11. Extra chromatin bands found on the tip of the left and right arm of the second chromosome and of the left arm of the third chromosome. *a*, normal type of IIL. *b-c*, heterozygous EIIIL. *d*, homozygous EIIIL. *e*, normal type of IIR. *f-g*, heterozygous EIIIL. *h*, homozygous EIIR. *i*, normal type of IIIL. *j-k*, heterozygous EIIIL. *l*, homozygous EIIIL. (Aceto-carmin preparations. \times ca. 1000).

thick band (divided into two in some preparations) and a thin band are attached to the distal end of the normal type chromosome (Fig. 11 *i, j, k, l*; Pl. II *i, j*).

It is interesting that these extra chromatin bands are detected always at the distal end of the chromosome. This, however, may be due to the fact that a small deficiency or duplication would be hard to detect if present in the middle portion of the chromosomes.

These chromosome aberrations have the following common characteristics: (1) They give no striking morphological or physiological peculiarity to the organism; (2) The flies with those chromosome aberrations in the homozygous state are viable; furthermore (3) so far as I have been able to ascertain, the distal portion of the chromosome in which one of such aberrations is present, never undergoes synapsis with any part of the same chromosome or of other chromosomes.

The first and second characteristics stated above seem to justify the view that the aberrations originated in duplications. The third characteristic, however, leads us to the supposition that the extra chromatins have been produced by the mutations of some "extant" genes. BRIDGES (1935) has already stated that the acquirement of new genic material is probably due to mutations of some genes involved in a duplicated piece — a phenomenon which is frequently observed under normal condition and is known as "repeat", an illustration of this is found in the left arm of the second chromosome of *D. melanogaster*.

An alternative explanation for the origin of the chromosome aberrations mentioned above is deficiency. However, the chromatin bands under consideration give no visible peculiarity either in the phenotype or in the fertility of the individual, even in the homozygous state. This condition is exceptional for deficiency. But, a similar case has recently been found by DEMEREC and HOOVER (1936) in *D. melanogaster*. The deficiency called 260-5 by them does not involve any of the visible extant loci in the distal end of the X-chromosome. Flies homozygous for the deficiency are viable and fertile, the only detectable effect of the deficiency is a decrease in the fertility of the female.

Thus, the question as to which of the two possibilities, duplication or deficiency, is more adequate for the explanation of the origin of such chromosome aberrations as detected in *D. ananassae*, is hard to decide at present. At any rate, it is of interest that there are several portions in the salivary chromosomes of this species which do not give any visible peculiarity to the organism.

FUTURE PROBLEMS

As stated already in the introduction of this paper, I have no intention of giving in detail my views on the results thus far obtained. The main problems to be reserved for future elucidation are:

(1) The tendency of occurrence of mutations in the same arms of the V-shaped chromosomes.

(2) The origin of the inert chromosomes and their rôle in the organisms.

(3) The origin of the sex chromosomes.

(4) The sexual difference in the crossover frequency in *Drosophila*.

(5) The occurrence of the same inversions in the same species as well as in related species.

(6) The origin and meaning of extra chromatin bands detected in salivary gland chromosomes.

In order to solve these problems thoroughly, investigations on more *Drosophila* species are much needed.

SUMMARY

(A) PART I

(1) *Taxonomical description*: *D. ananassae* is one of the tropical species distributed widely in southern states of North America, Central America, South America, South Sea Islands, Ryukyu, Formosa and southern regions of China.

(2) *Chromosomes*: In the oogonial metaphase or in the division of a nerve cell of the female, there are found four pairs of V-shaped chromosomes, one pair of which is relatively shorter than the others. In the male, one chromosome of a larger V-shaped pair (i.e. X pair) is replaced by a small J-shaped Y-chromosome. In the salivary gland nucleus, only six strands which correspond to the two arms of the first, second and third chromosomes respectively, radiate from the chromocenter. The remaining two strands which represent the two arms of the fourth chromosome, exist in the chromocenter as heterochromatic masses.

(3) *Mutants and Linkage maps*: 71 mutants are described briefly with available maps (Fig. 5). Of these, *Plum* and *bobbed-IV* factors are described in some detail.

(B) PART II

(1) In *D. ananassae*, there is a tendency for mutations to occur in the same arm of V-shaped chromosomes.

(2) The shortest pair of the germinal chromosomes (i.e., the fourth chromosomes) are virtually inert; only one mutation *bobbed-IV* has hitherto been found in this pair.

(3) The distal part of the longer arm of this inert chromosome is homologous to one arm of the Y-chromosome. The nucleolus-forming region and the *bobbed-IV* (*bobbed*) gene are probably located in this part. It is assumed, moreover, that a part of the inert chromosome is homologous to a part of the X-chromosome.

(4) When an enhancer (or enhancers) of crossing over is introduced in a strain, male crossing over occurs rather frequently in the normal condition.

(5) There are found five kinds of inversions in wild populations of *D. ananassae*; three of these, CIIL, CIIL, CIIIR, are distributed widely in the strains of such distant localities as North America, Japan and China (Fig. 10; Pls. I and II); the other two inversions have been detected each in a single strain.

(6) Some of the salivary gland chromosomes of several strains have one or a few additional bands attached to the tip ends. They are called EIIL, EIIR and EIIIL according to the number of chromosome strands (Fig. 11; Pl. II *g-j*).

Lastly it has been pointed out that the observations stated above are of significance in the problems of evolution of species of *Drosophila*.

APPENDIX I

LEGENDS FOR SYMBOLS, MUTATIONS, VALUATIONS

In the following list symbols of all mutations of *D. ananassae*, including other symbols used in this paper, are arranged alphabetically. A complete listing contains: symbol, full name, chromosome and locus, reference figure in this paper, published account, description and valuation, and the record or reference.

Abbreviation of references

M34 — MORIWAKI, D., 1934. Jap. Journ. Genet. 9; 164-168.

M35a — MORIWAKI, D., 1935 a. Genetica 17; 32-46.

- M35b — MORIWAKI, D., 1935b. Jap. Journ. Genet. 11; 302-307.
 M35d — MORIWAKI, D., 1935d. Proc. Imp. Acad. Tôkyo 11; 340-341.
 M36a — MORIWAKI, D., 1936a. Zool. Mag. Tôkyo 48; 285-286
 M36b — MORIWAKI, D., 1936b. Jap. Journ. Genet. 12; 183-188.
 M36c — MORIWAKI, D., 1936c. Zool. Mag. Tôkyo 48; 693-701.
 M37a — MORIWAKI, D., 1937a. Jap. Journ. Genet. 13; 4.
 M37b — MORIWAKI, D., 1937b. Cytologia (FUJII Jubilai Vol.); 228-233.

ab — abnormal (I-163.6) (Pl. VI d). In the male carrying this character, the external genitalia are rotated from 30° to 180°. Sometimes they are rudimentary and no opening is observed in the hypopygium, but testes are usually normal. Male, sterile. Rk 4. Lost.

ac — achaete (I-102.0) (M36c, Fig 11). All bristles except a few missing. Rk 1.

ba — balloon (III-35.0 ±) (Pl. IVf; Pl. VI f) (M34; M35a, Fig 12; M36c, Fig. 4). Wings are inflated and slightly extended. This character is occasionally associated with black vesicles in the wings. Sometimes the body shows blackish color, especially at low temperature. Rk 2 at 20° C, Rk 5 at 30° C. Lost. 35b15 (KIKKAWA).

bb-IV or *bb* — bobbed-IV or bobbed (IV-0.0) (Pl. VI g) (M35d; M36c, Fig. 7; M37ab). See Part I, Chapter III of this paper. Rk. 2.

*bb*²-IV — bobbed²-IV Allele of *bb*. Slightly less extreme than *bb* Rk 2.

Bb — Barb (III-39.5) (Pl. VII b). Bristles with a knot at tip. Bristles and hairs are somewhat longer than those of normal type. Lethal when homo. Rk 2.

bn — broken (III-22.0 ±) (Pl. IVd). Posterior crossvein missing or interrupted. Wings are slightly rounded and down-curved. Rk 1 at 27° C, Rk 3 at 20° C.

br — broad (I-72.0) (Pl. III e). In the male, wing about 2/3 of normal length, with blunt tip. Crossveins close together. In the female, the expression of the character is relatively suppressed. Rk 1 in ♂, Rk 3 in ♀.

c — curved-wing (A-?). Wings down-curved. Lost

C — Symbol of Inversion.

c-IIa — curved-IIa (II-1.0?) (Pl. VII a). In the female, wing and abdomen down-curved. Hairs on abdomen are somewhat erected at the base of each segment. In the male, no such characters are observed. But at low temperature, wings are often down-curved. Rk 2 at 23° C, Rk 5 at 30° C. Lost.

c-IIb — curved-IIb (II-?). Wings down-curved. Rk 2.

c-IIIa — curved-III a (III-27.5 ±). Wings down-curved. Rk 2. Lost.

cd — cardinal (II-0.0). A vermilion eye color that tends to overlap wild type, especially when old. Ocelli white. Rk 1.

ck — crooked (I-?). (M34; M35a, Fig. 10; M36c, Fig. 13). Tarsal joints of all legs, especially of metathoracic legs, are crooked. Overlaps wild type. Rk 5. Lost.

cl — crumpled (I-117.6 ±). (M35b; M36c, Fig. 2). Wrinkled wings associate with the cut character. In the male, the expression is reduced considerably. Semilethal. Probably an extreme allele of cut.

cr — crumpled (A-?). (M34; M35a; M36c, Fig. 2). Wings crumpled. Body color brownish. Eyes somewhat dark and rough. Bristles irregular in direction. Lost.

ct — cut (I-117.6) (Pl. IVa; Pl. VI e). Edge of wing scalloped. Antennae flattened, arista concave forward. They show often the forms of three distal tarsal joints. Rk 2 at 23° C, Rk 4 at 30° C.

*ct*² — cut² (Pl. IVb). Allele of *ct*. Marginal vein and edge of wing scalloped. Arista normal. Females of the constitution *ct/ct*² show the following characteristics: (1) costal vein is not scalloped in general, (2) notches of the edge of wing are not so extreme as those of *ct*. Thus the two alleles seem to act as suppressors of each other. Rk 1.

dwp — dwarf (I-0.0) (Pl. VI b). Body about $\frac{2}{3}$ of normal size. Wings warped and somewhat extended. Bristles relatively short. Male, sterile. Rk 4. Lost.

dy — dusky (I-46.2 \pm) (Pl. Vh). Wings about $\frac{2}{3}$ of normal length, with blunt tips. They are slightly dusky in color. Eyes larger than normals, and somewhat irregular. Rk 1.

e — ebony (II-49.1). Black body color. Veins also more blackish than normals. Heterozygous flies are often differentiated from normal ones. Rk 1.

E — Symbol of extra chromatin bands found in salivary gland chromosomes.

En — Enhancer or Enhancers (III-?). Enhancers of crossing over in the male.

et — extra (III-36.5 \pm) (Pl. IVg). There is generally an extra vein between two crossveins, which is interrupted in the middle part. But sometimes only the anterior cross vein is disarranged. Wings are somewhat shortened and broadened. Eyes are slightly larger than normal. Semidominant. Rk 1.

ex — extended (II-?) (M36c, Fig. 16). Wings extended. Various manifestations. Rk 4.

f — forked (I-41.5) (Pl. VII f). Posterior scutellars and a few other bristles forked or twisted. Rk 2.

*f*² — forked² (Pl. VI a). Allele of *f*. More extreme than *f*. All bristles and hairs twisted and gnarled. Rk 1.

g — garnet (I-37.5). A yellowish pink eye color. Body slightly yellowish in color. Rk 1.

*g*² — garnet². Allele of *g*. A dark pinkish eye color. The order of dominance is: + > *gf* > *g*. Rk 1.

gp — gap (III-33.5 \pm) (Pl. IV e). The fourth longitudinal vein is broken beyond posterior crossvein. The posterior crossvein is somewhat oblique in direction. Semidominant. Rk 1.

H-IV — Haplo-IV or Diminished (*Dm*) (IV) (Fig. 6). See Part II, Chapter II of this paper. Lethal when homo. Rk 3.

ic — incomplete (A) (M34, Fig. 1; M35a, Fig. 2; M36c, Fig. 1). The fourth longitudinal vein is short, not reaching the marginal vein. Lost.

Ir — Interrupted (I-109.6 \pm). (M34, Fig. 2; M35a, Figs. 3, 4, 5; M36c, Fig. 3). Posterior crossvein missing or interrupted. Longitudinal veins thickened at tips. Homo, viable. Rk 4. 33e12 (Moriwaki).

L — Symbol of the left arm of each chromosome.

l-1 — lethal-1 (I-?) (M35a; M36c). A gene for viability. Lost.

l-2 — lethal-2 (II-?) (M35a; M36c). A gene for viability. Lost.

l-3 — lethal-3 (I-?) (M36c). A gene for viability.

l-IIIa — lethal-IIIa (III-35.0 \pm). A gene for viability. Balanced with Plum. Rk 2.

la — lance (II-?). Narrow pointed wing. Semidominant. Rk 4. Lost.

ll — lanceolate (III-?). Narrow pointed wing. Rk 4.

m — miniature (I-45.5) (Pl. III f). Miniature wing being about $\frac{2}{3}$ of normal size. It is somewhat dusky in color. Rk 1.

M-Ia — Minute-Ia (I-30.5). Bristles small. Hatches about one day later than normals. Lethal when homo. Rk 2.

M-IIa — Minute-IIa (II-74.0?). Bristles small. Lethal when homo. Rk 3. Lost.

M-IIb — Minute-IIb (II-?) (M37ab). Bristles small. It is associated with an enhancer of crossing over in both sexes. Lethal when homo.

M-IIc — Minute-IIc (II-?) Bristles small. Lethal when homo. Rk. 3. Lost.

M-IIIa — Minute-IIIa (III-19.0 \pm). Bristles small. Lethal when homo. Rk 3. Lost.

M-IIIb — Minute-IIIb (III-26.5). Bristles small. Lethal when homo. Rk 2.

M-IIIc — Minute-IIIc (III-?). (M36c, Fig. 10). Bristles small. Lethal when homo.

ms — missing (II-56.7 \pm) (M36c, Fig. 9). Bristles, especially scutellars often missing. Rk 4.

N — Notch (I-15.5) (Pl. III d). Wings have terminal notches, usually at tips of III and IV longitudinal veins. III and V longitudinal veins are thickened throughout. Eyes slightly small and rough. Rk. 2.

ob — obliterated (II-?). Posterior crossvein missing or interrupted. Rk 4.

Off — Off (II-37.8) (M36c, Fig. 12). Bristles reduced and gnarled. Homo, viable. Rk 1. Lost.

ph — purplish (I-65.0) (M36a; M36c). Dark purplish eye color. Rk 1.

pk — prickly (II-60.6). Bristles cut off like stubbles. Rk 1.

Pm — Plum (III-35.0). See Part I, Chapter III of this paper. Rk 1

Pt — Plexate (II-23.6) (Pl. VI c) (M34; M35a, Figs. 6, 7; M36b; M36c, Fig. 5). Longitudinal veins, especially the second, expand into deltas where they join margin. Variable manifestations. In combination with *px* (III) the Plexate character is much exaggerated. Lethal when homo. Rk 1. 34b25 (KIKKAWA), 35j20 (KIKKAWA).

Pt¹ — Plexate¹ Allele of *Pt*. Less extreme than *Pt*. Only the second longitudinal vein expands into delta where it joins margin. Rk 2. Lost.

px — plexus (III-83.5) (Pl. IV h). Extra and branched veins, especially in the submarginal and the third posterior cell. Rk 1.

R — Symbol of the right arm of each chromosome.

rt — retracted (II-?) (M34; M35a, Fig. 8; M36c, Fig. 6). Wings are markedly shortened and rounded. Overlaps wild type. Rk 3.

- sb* — stubble (II-?) (Pl. VII *d*). Short bristles like stubbles. Orbital, dorso-central and postalar bristles are often missing. Rk 3. Lost.
- sc* — scute (I-96.0) (M36c, Fig. 8). Orbitals (A.M.P.), posterior notopleural, anterior, postalar and scutellars (A.P.) missing. Occasionally anterior supraalar missing. Rk 1.
- sd* — spread (II-20.3 \pm) (Pl. VI *c*). Wings spread. There are often blackish spots on coxa, trochanter of each leg, especially of protoracic leg. Rk 3. Lost.
- sk* — ski (II-23.6 \pm) (M34; M35a, Fig. 11; M36c, Fig. 14). Wings upturned. Overlaps wild type. Rk 4.
- sk-III* — ski-III (III-0.0) (M36c, Fig. 15). Tips of wings curled upward like ski. Rk 3.
- sl* — slender (II-60.0 \pm). Bristles slender and short. A few dorsocentral and scutellar bristles often missing. Rk 3.
- sn* — singed (I-112.7) (Pl. VII *g*). All bristles slightly twisted and gnarled. Hairs normal. Rk 1.
- sn²* — singed² (Pl. VII *h*). Allele of *sn*. More extreme than *sn*. All bristles and hairs twisted and gnarled. Rk 1.
- ss* — spineless (II-24.5) (Pl. VII *e*). Bristles and hairs are very short and slender. Sclerites occasionally scaly. Eye color somewhat darker than normal. Rk 2.
- tb* — tiny-bristle (II-39.5) (Pl. VII *c*). All bristles slender and short. Rk 1.
- TR* — Symbol of translocation.
- v* — vermilion (I-44.0). Vermilion eye color, not translucent. Rk 1. 36c20 (KIKKAWA), 37c16 (KIKKAWA).
- w* — white (I-16.7) (M34; M35a; M36c). Snowy white eye-color. Rk 1. 34i18 (KIKKAWA), 35a26 (KIKKAWA), 35j15 (KIKKAWA).
- w^a* — apricot. Allele of *w*. Light pinkish yellow eye color. In young imago, it is very light. The order of dominance: + > *w^a* > *w*. Rk 1.
- X* — Symbol of the X-chromosome
- y* — yellow (I-96.2) (M36c). Yellow body color. Veins and bristles as well as hairs are also yellow. Rk 1. 36b21 (KIKKAWA).
- Y* — Symbol of the Y-chromosome
- I, II, III, IV* showing the number of the first, second, third and fourth chromosomes respectively.
- + — Symbol of wild type. (Fig. 1; Pl. III *c*; Pl. V *e, g*). (M35a, Figs. 1, 9; M36c).

APPENDIX II

SUMMARY OF LINKAGE DATA ON *D. ananassae*

In the following table are given the summarized linkage data obtained by the present author. For comparison's sake, available data taken from MORIWAKI's paper (Zool. Mag. Tōkyō 48, 693-701, 1936) are shown at the end.

THE FIRST CHROMOSOME

Loci tested	Crossovers	Total flies	Recombination percentage
<i>dp</i> — <i>v</i>	296	674	43.9
<i>dp</i> — <i>ct</i>	295	614	48.0
<i>N</i> — <i>w</i>	25	2002	1.2
<i>N</i> — <i>g</i>	207	944	21.9
<i>N</i> — <i>f</i>	51	171	29.8
<i>N</i> — <i>m</i>	78	269	29.0
<i>N</i> — <i>ph</i>	188	444	42.3
<i>w</i> — <i>M-Ia</i>	69	713	12.5
<i>w</i> — <i>f</i>	1100	4342	25.3
<i>w</i> — <i>m</i>	609	2175	28.0
<i>w</i> — <i>dy</i>	37	129	28.7
<i>w</i> — <i>br</i>	1697	4692	36.2
<i>w</i> — <i>sc</i>	245	559	43.8
<i>w</i> — <i>ac</i>	221	433	51.0
<i>w</i> — <i>sn</i>	119	239	49.8
<i>w</i> — <i>ct</i>	586	1229	47.7
<i>w</i> — <i>ab</i>	67	129	51.9
<i>M-Ia</i> <i>br</i>	28	91	30.8
<i>g</i> — <i>f</i>	48	1243	3.9
<i>g</i> — <i>v</i>	58	1304	4.4
<i>g</i> — <i>m</i>	171	2235	7.7
<i>g</i> — <i>br</i>	42	115	36.5
<i>g</i> — <i>lt</i>	42	100	42.0
<i>g</i> — <i>sn</i>	441	948	46.5
<i>g</i> — <i>ct</i>	211	424	49.8
<i>g</i> — <i>ab</i>	105	214	49.1
<i>f</i> — <i>v</i>	21	858	2.4
<i>f</i> — <i>m</i>	56	1204	4.7
<i>f</i> — <i>dy</i>	7	129	5.4
<i>f</i> — <i>ph</i>	129	554	23.3
<i>f</i> — <i>br</i>	936	3810	24.6
<i>f</i> — <i>ct</i>	64	132	48.5
<i>f</i> — <i>ab</i>	304	634	47.9
<i>v</i> — <i>m</i>	6	446	1.4
<i>m</i> — <i>ph</i>	40	213	18.8
<i>m</i> — <i>lt</i>	42	100	42.0
<i>m</i> — <i>sn</i>	540	1187	45.5
<i>m</i> — <i>ct</i>	346	783	44.2

Loci tested	Crossovers	Total flies	Recombination percentage
<i>ph — br</i>	38	554	6.9
<i>ph — sc</i>	648	2154	30.1
<i>ph — y</i>	166	539	30.8
<i>ph — ct</i>	1412	3284	43.0
<i>br — sc</i>	134	559	24.0
<i>br — ac</i>	125	433	28.9
<i>br — ct</i>	273	732	37.3
<i>br — ab</i>	208	425	48.9
<i>sc — y</i>	1	539	0.2
<i>sc — ct</i>	414	1615	25.6
<i>y — sn</i>	69	418	16.5
<i>y — ct</i>	81	418	19.4
<i>Ir — ct</i>	8	100	8.0
<i>sn — ct</i>	53	1090	4.9
<i>ct — ab</i>	99	215	46.0

THE SECOND CHROMOSOME

<i>cd — Pt</i>	1118	4743	23.6
<i>cd — ss</i>	188	612	30.7
<i>cd — tb</i>	371	1161	32.0
<i>cd — e</i>	177	446	39.6
<i>cd — ph</i>	373	804	46.4
<i>cd — sl</i>	193	442	43.7
<i>cd — M-IIa</i>	233	485	48.0
<i>c-IIa — Pt</i>	26	114	22.8
<i>sd — Pt</i>	2	61	3.3
<i>Pt — ss</i>	9	960	0.9
<i>Pt — tb</i>	310	1951	15.9
<i>Pt — e</i>	223	874	25.5
<i>Pt — ph</i>	407	1101	37.0
<i>Pt — sl</i>	219	519	42.2
<i>Pt — M-IIa</i>	173	344	50.3
<i>sh — Pt</i>	191	414	46.1
<i>M-IIc — cd</i>	108	227	47.6

THE THIRD CHROMOSOME

<i>sk-III — Pm</i>	81	236	34.3
<i>sk-III — Bb</i>	124	311	38.9
<i>M-IIIa — ba</i>	136	858	15.9

Loci tested	Crossovers	Total flies	Recombination percentage
<i>M-IIIa — px</i>	207	441	46.9
<i>bn — M-IIIb</i>	31	695	4.5
<i>bn — Pm</i>	84	630	13.3
<i>bn — Bb</i>	19	136	14.0
<i>M-IIIb — c-IIIa</i>	4	364	1.1
<i>M-IIIb — gp</i>	16	194	8.3
<i>M-IIIb — ba</i>	14	199	7.0
<i>M-IIIb — Pm</i>	6	70	8.6
<i>M-IIIb — et</i>	8	70	11.4
<i>M-IIIb — Bb</i>	8	94	8.5
<i>M-IIIb — px</i>	403	908	44.4
<i>gp — Pm</i>	8	573	1.4
<i>gp — Bb</i>	45	547	8.2
<i>ba — Pm</i>	0	286	0.0
<i>ba — Bb</i>	39	763	5.1
<i>Pm — et</i>	12	793	1.5
<i>Pm — Bb</i>	4	88	4.5
<i>Pm — px</i>	644	1366	47.1
<i>Bb — px</i>	640	1454	44.0
<i>Bb — ll</i>	30	84	38.1

LINKAGE DATA OBTAINED BY MORIWAKI

THE FIRST CHROMOSOME

Loci tested	Total flies	Recombination percentage
<i>w — f</i>	2943	25.4
<i>f — v</i>	619	9.0
<i>f — m</i>	1065	9.6
<i>f — ph</i>	800	21.8
<i>ph — sc</i>	479	25.7
<i>ph — ct</i>	800	43.4
<i>br — sc</i>	434	29.9
<i>sc — y</i>	227	1.8
<i>sc — ac</i>	107	11.2
<i>sc — et</i>	479	31.9
<i>ct — cl</i>	2143	0.0

THE SECOND CHROMOSOME

Loci tested	Total flies	Recombination percentage
<i>cd — Pt</i>	4147	25.9
<i>cd — ss</i>	4147	26.5
<i>cd — Off</i>	535	34.4
<i>Pt — sk</i>	1171	0.0
<i>Pt — ss</i>	4147	0.8
<i>Pt — Off</i>	268	14.2
<i>Pt — ms</i>	154	33.1
<i>Off — ms</i>	141	28.4

THE THIRD CHROMOSOME

<i>sk-III — M-IIIb</i>	391	37.3
<i>sk-III — Bb</i>	334	42.5
<i>M-IIIc — ba</i>	273	46.9
<i>M-IIIc — px</i>	381	50.1

APPENDIX III

THE SUMP METHOD

This method devised by Dr. J. SUZUKI, is used for examining the surface of an opaque body. In this method, the test-material, such as the abdomen of *Drosophila*, is put on the surface of a celluloid-piece softened by amyl-acetate. After several minutes, the material is removed from the surface, and the celluloid-piece brought under a common microscope. In this way, a beautiful image of the test-material is obtained. The real value of this method may be readily appreciated from the examples shown in Plate V *e, f, g, h*.

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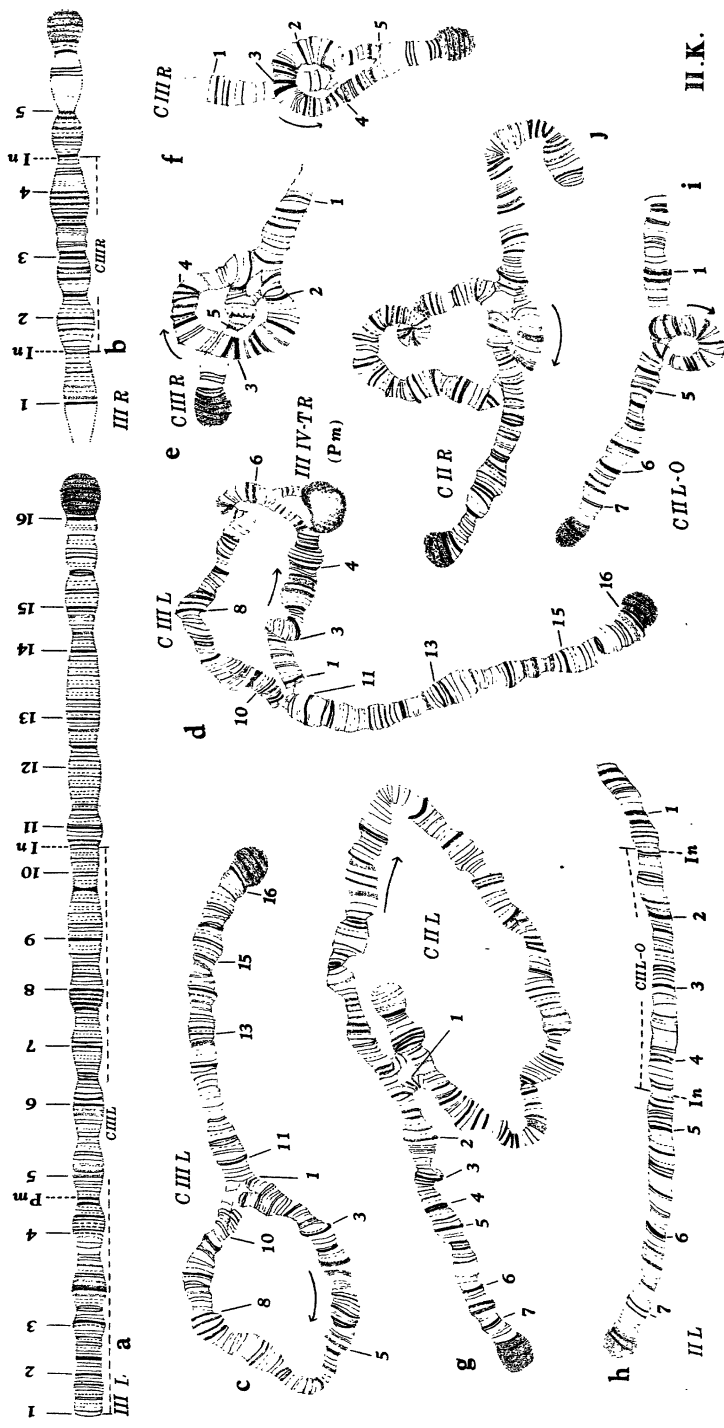
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Camera lucida drawings of salivary chromosomes of *D. ananassae* (Aster-carminae preparations. \times ca 1000).

a. Schematic figure of the left arm of third chromosome (A type) in a salivary gland nucleus.

b. The same of the proximal portion of the right arm of third chromosome.

c. A terminal inversion (CIII L) found in the left arm of third chromosome (Okinawa-T strain).

d. The same in the case where a III-IV mutual translocation is involved (Phum stock).

e. A basal inversion (CIII L) found in the right arm of third chromosome (Tokyo-M strain).

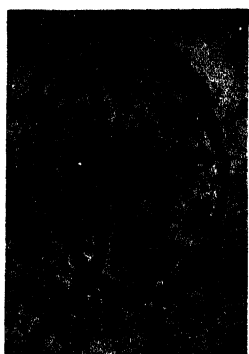
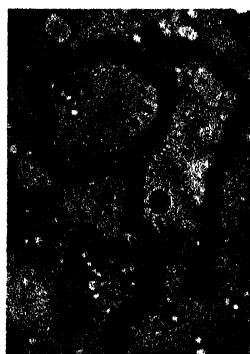
f. The same (Naka-Iwoto-D strain).

g. A subterminal inversion (CIII L) found in the left arm of second chromosome (Okinawa-T strain).

h. Figure showing the proximal portion of the left arm of second chromosome.

i. A basal inversion (CIII-O) found in the proximal portion of the left arm of second chromosome (Tahoku-O strain).

j. A median inversion (CIII R) found in the right arm of second chromosome (Tusatsosa-K strain).

*a**b**c**d**e**f**g**h**i**j*

Microphotographs of salivary chromosomes of *D. ananassae* (Acetocarmine preparations. *a-f*, \times ca. 350. *g-j*, \times ca. 400).

- a*, A subterminal inversion (CIIL) found in the left arm of second chromosome (Shanghai-H strain).
- b*, A median inversion (CIIR) found in the right arm of second chromosome (Tuscaloosa-K strain).
- c*, A terminal inversion (CIIL) found in the left arm of third chromosome (Tuscaloosa-K strain).
- d*, The same (Shanghai-H strain).
- e*, A basal inversion (CIIR) found in the right arm of third chromosome (Tokyo-M strain).
- f*, The same (Naka-Iwôtô-D strain).
- g*, The distal portion of the left arm of second chromosome (Normal type) (Tuscaloosa-K strain).
- h*, The same in the case where extra chromatin bands (EIL) are present in a heterozygous state (Shanghai-H strain).
- i*, The distal portion of the left arm of third chromosome (Normal type) (Shanghai-H strain).
- j*, The same in the case where extra chromatin bands (EIL) are present in a homozygous state (Shanghai-T strain).



a



b



c



d



e



f



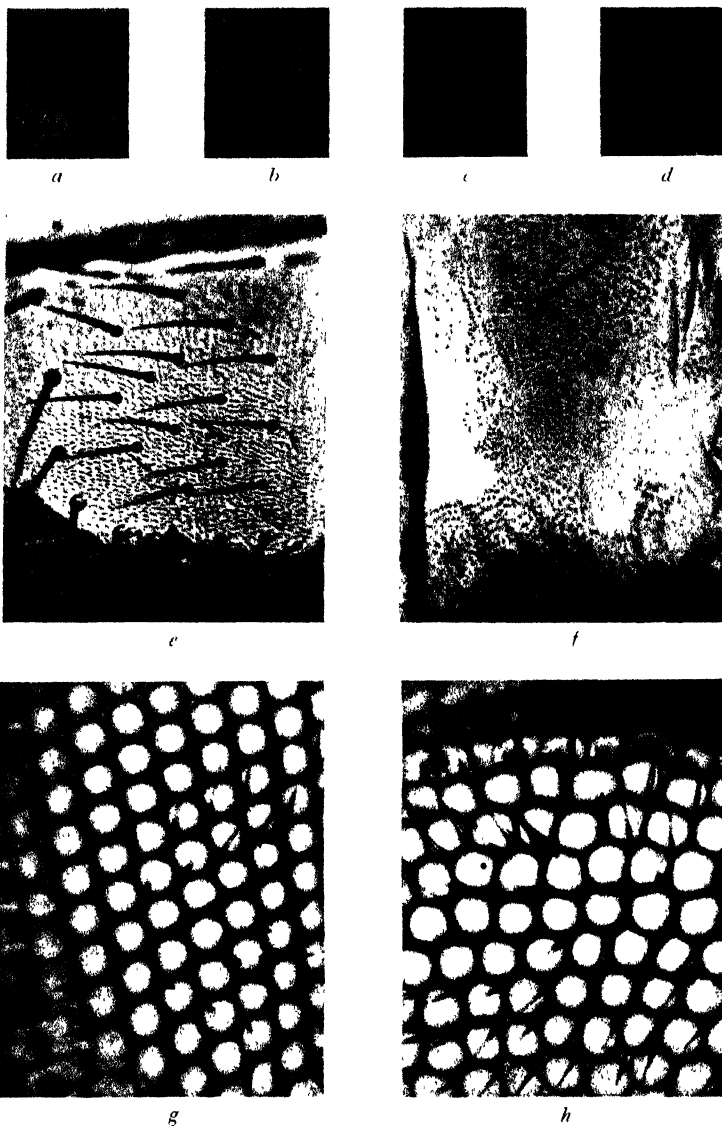
g



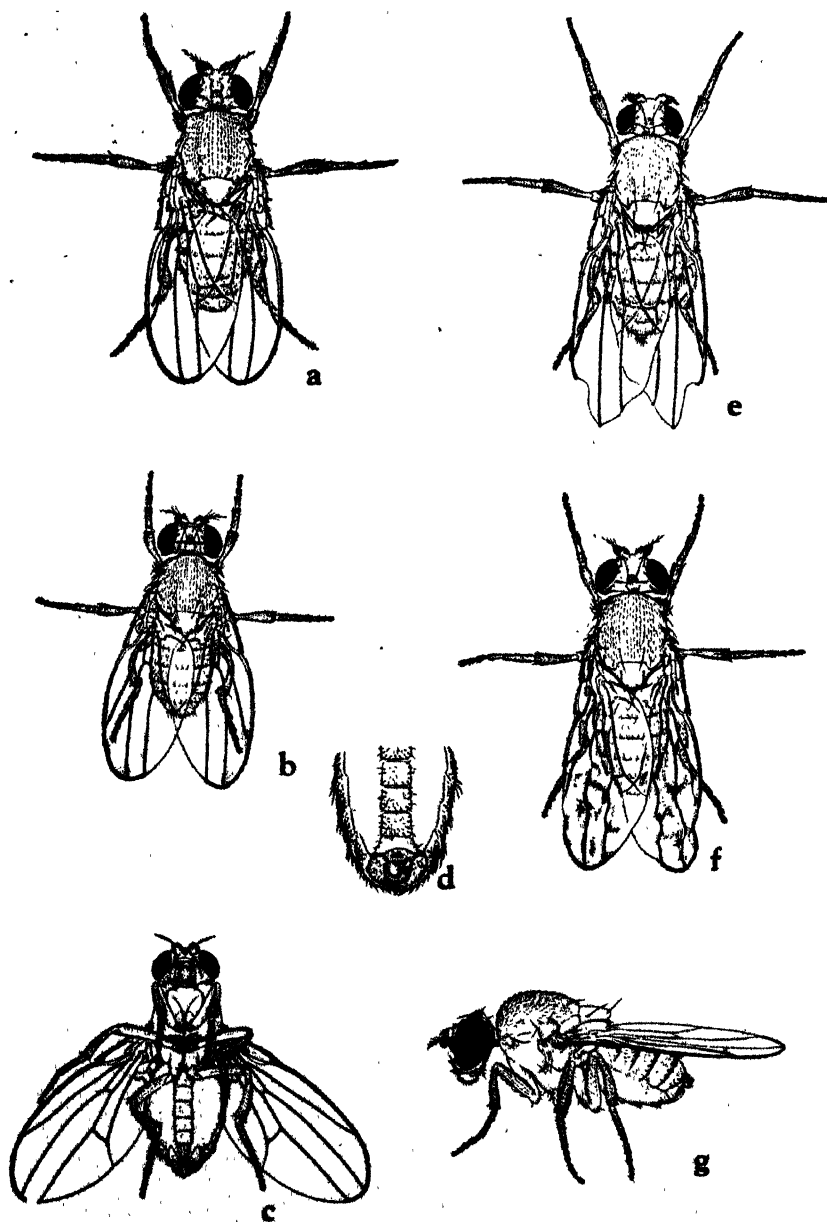
h

Microphotographs of wing mutations. (*a-h*, \times ca. 16).

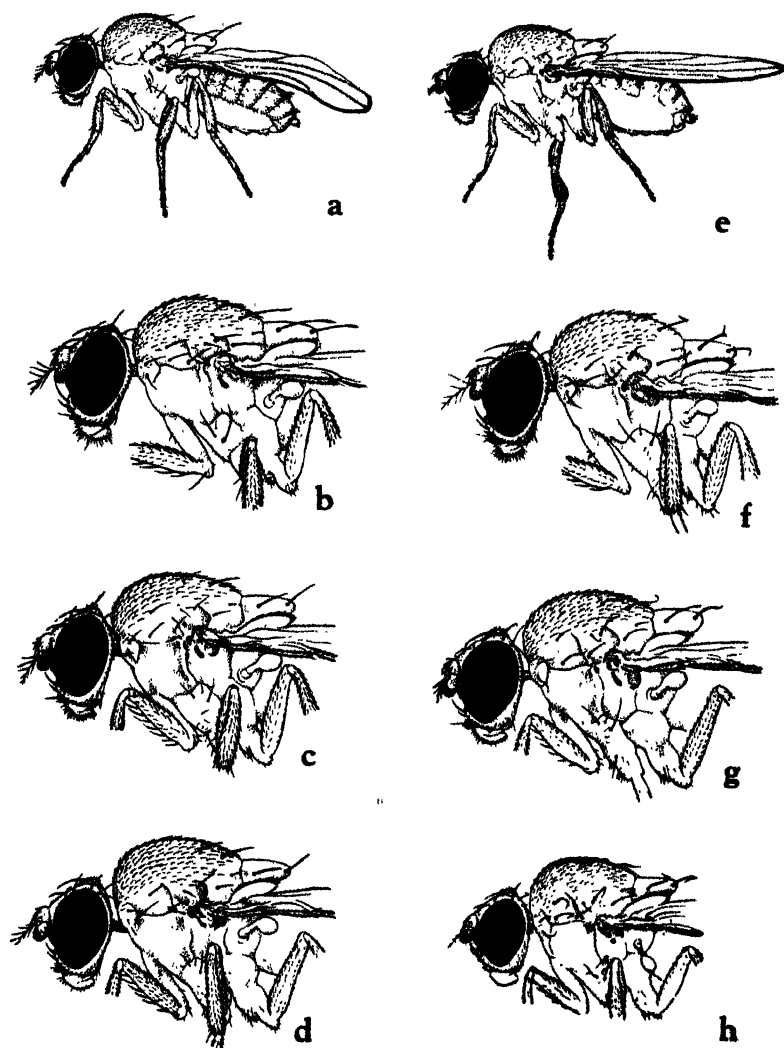
a, cut (I). - *b*, cut² (I). - *c*, Plexate (II). - *d*, broken (III). - *e*, gap (III). - *f*, balloon (III). - *g*, extra (III) - *h*, plexus (III).



Microphotographs of oögonial chromosome, abdomens and eyes. (*a-d* aceto-carmin preparations. \times ca. 2000. *e-h*, Sump preparations \times ca. 100. See Appendix III) - *a*, XX haplo-IV type (Fig. 2 *d*). - *b*, XXY haplo-IV type (Fig. 2 *f*). - *c*, XXY type (Fig. 2 *h,i*). - *d*, XX triplo-IV type (Fig. 2 *j*). - *e*, Abdomen of wild type. - *f*, The same of bobbed. - *g*, Eye surface of wild type. - *h*, The same of dusky.



Figures of mutant characters. *d*, \times ca. 25, others \times ca. 15.
a, forked² (I). - *b*, dwarf (I). - *c*, spread (II). - *d*, abnormal (I).
e, cut (I). - *f*, balloon (III). - *g*, bobbed-IV (IV).



Figures of mutant characters. *a, e*, \times ca. 15, others \times ca. 25.

a, curved-IIa (II). - *b*, Barb (III). - *c*, tiny-bristle (II). - *d*, stubble (II). - *e*, spineless (II). - *f*, forked (I). - *g*, singed (I). - *h*, singed¹ (I).

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